

ANNALS OF BOTANY

EDITED BY

V. H. BLACKMAN, Sc.D., F.R.S.

EMERITUS PROFESSOR OF PLANT PHYSIOLOGY, IMPERIAL
COLLEGE OF SCIENCE AND TECHNOLOGY, LONDON

ASSISTED BY

A. J. EAMES, Ph.D.

PROFESSOR OF BOTANY, CORNELL UNIVERSITY
ITHACA, N.Y., U.S.A.

AND OTHER BOTANISTS

NEW SERIES. VOLUME IX

With six Plates, and two hundred and twenty-three Figures

1945


Reprinted by

Wm. DAWSON & SONS LTD.,

LONDON

with the permission of the Publishers

1963



Digitized by the Internet Archive
in 2024

ANNALS OF BOTANY

EDITED BY

V. H. BLACKMAN, Sc.D., F.R.S.

EMERITUS PROFESSOR OF PLANT PHYSIOLOGY, IMPERIAL
COLLEGE OF SCIENCE AND TECHNOLOGY, LONDON

ASSISTED BY

A. J. EAMES, Ph.D.

PROFESSOR OF BOTANY, CORNELL UNIVERSITY
ITHACA, N.Y., U.S.A.

AND OTHER BOTANISTS

NEW SERIES. VOLUME IX

With six Plates, and two hundred and twenty-three Figures

1945

Reprinted by

Wm. DAWSON & SONS LTD.,

LONDON

with the permission of the Publishers

1963

OXFORD UNIVERSITY PRESS

AMEN HOUSE, E.C. 4

LONDON EDINBURGH GLASGOW NEW YORK

TORONTO MELBOURNE CAPE TOWN BOMBAY

CALCUTTA MADRAS

GEOFFREY CUMBERLEGE

PUBLISHER TO THE UNIVERSITY

CONTENTS

1127-10

Volume IX, No. 33, January 1945

FRITSCH, F. E. Studies in the Comparative Morphology of the Algae. IV. Algae and Archegoniate Plants. With twenty-one Figures in the Text	I
MILTHORPE, F. L. Fibre Development of Flax in Relation to Water Supply and Light Intensity. With eight Figures in the Text	31
BOSWELL, J. G. Oxidation Systems in the Potato Tuber. With six Figures in the Text	55
BARNELL, H. R., and BARNELL, E. Studies in Tropical Fruits. XVI. The Distribution of Tannins within the Banana and the Changes in their Condition and Amount during Ripening. With nine Figures in the Text.	77

No. 34, April 1945

GOODALL, D. W. The Distribution of Weight Change in the Young Tomato Plant. I. Dry-weight Changes of the Various Organs. With five Figures in the Text.	101
DORMER, K. J. An Investigation of the Taxonomic Value of Shoot Structure in Angiosperms with Especial Reference to Leguminosae. With nine Figures in the Text	141
MANTON, IRENE. Chromosome Length at the Early Meiotic Prophases in Osmunda. With Plates I and II, and forty-nine Figures in the Text	155
SWAMY, B. G. L., and THIRUMALACHAR, M. J. Homologies of the Embryo Sac of Angiosperms. With one Figure in the Text	179
BOND, T. E. T. Studies in the Vegetative Growth and Anatomy of the Tea Plant (Camellia thea Link.) with Special Reference to the Phloem. II. Further Analysis of Flushing Behaviour. With thirteen Figures in the Text	183

No. 35, July 1945

WARDLAW, C. W. Experimental and Analytical Studies of Pteridophytes. V. Stelar Morphology: The Development of the Vascular System. With twenty-eight Figures in the Text.	217
HATCHER, E. S. J. Studies in the Vernalisation of Cereals. IX. Auxin Production during Development and Ripening of the Anther and Carpel of Spring and Winter Rye. With four Figures in the Text	235
DARLINGTON, C. D., and JANAKI-AMMAL, E. K. Adaptive Isochromosomes in Nicandra. With ten Figures in the Text	267
LAL, B. N. Plant-Injection Methods for the Diagnosis of Mineral Deficiencies in Tobacco and Soya Bean. With Plate III and eight Figures in the Text	283

No. 36, October 1945

PHILLIS, E., and MASON, T. G. Studies on Foliar Hydration in the Cotton Plant. VI. A 'Gel Theory of Cell Water Relations. With ten Figures in the Text	297
MASON, T. G., and PHILLIS, E. Studies on the Partition of the Mineral Elements in the Cotton Plant. V. An Adsorption Theory of Nitrogen Regulation. With one Figure in the Text	335
MASON, T. G., and PHILLIS, E. The Effect of Ringing and of Transpiration on Mineral Uptake: A Reply to Criticism. With one Figure in the Text	345

PHILLIS, E., and MASON, T. G. The Effect of Extreme Desiccation on the Viability of Cotton Seed. With four Figures in the Text	353
HUTCHINSON, J. B., STEPHENS, S. G., and DODDS, K. S. The Seed Hairs of <i>Gossypium</i> . With Plate IV	361
DE ROPP, R. S. Studies in the Physiology of Leaf Growth. I. The Effect of Various Accessory Growth Factors on the Growth of the first Leaf of Isolated Stem Tips of Rye. With three Figures in the Text	369
WARDLAW, C. W. Experimental and Analytical Studies of Pteridophytes. VI. Stelar Morphology: The Occurrence of Reduced and Discontinuous Vascular Systems in the Rhizome of <i>Onoclea sensibilis</i> . With Plates V and VI and thirty-three Figures in the Text	383

LIST OF PLATES

- I, II. Chromosome Length (MANTON).
- III. Plant Injection (LAL).
- IV. Seed Hairs of *Gossypium* (HUTCHINSON, STEPHENS, and DODDS).
- V, VI. *Onoclea sensibilis* (WARDLAW).

INDEX OF CONTRIBUTORS

BARNELL, E., see BARNELL, H. R.	
BARNELL, H. R., and BARNELL, E. Studies in Tropical Fruits. XVI. The Distribution of Tannins within the Banana and the Changes in their Condition and Amount during Ripening. With nine Figures in the Text	77
BOND, T. E. T. Studies in the Vegetative Growth and Anatomy of the Tea Plant (<i>Camellia thea</i> Link.) with Special Reference to the Phloem. II. Further Analysis of Flushing Behaviour. With thirteen Figures in the Text	183
BOSWELL, J. G. Oxidation Systems in the Potato Tuber. With six Figures in the Text	55
DARLINGTON, C. D., and JANAKI-AMMAL, E. K. Adaptive Isochromosomes in <i>Nicandra</i> . With ten Figures in the Text	267
DE ROPP, R. S. Studies in the Physiology of Leaf Growth. I. The Effect of Various Accessory Growth Factors on the Growth of the first Leaf of Isolated Stem Tips of Rye. With three Figures in the Text	369
DODDS, K. S., see HUTCHINSON, J. B.	
DORMER, K. J. An Investigation of the Taxonomic Value of Shoot Structure in Angiosperms with Especial Reference to Leguminosae. With nine Figures in the Text	141
FRITSCH, F. E. Studies in the Comparative Morphology of the Algae. IV. Algae and Archegoniate Plants. With twenty-one Figures in the Text	1
GOODALL, D. W. The Distribution of Weight Change in the Young Tomato Plant. I. Dry-weight Changes of the Various Organs. With five Figures in the Text	101
HATCHER, E. S. J. Studies in the Vernalisation of Cereals. IX. Auxin Production during Development and Ripening of the Anther and Carpel of Spring and Winter Rye. With four Figures in the Text	235
HUTCHINSON, J. B., STEPHENS, S. G., and DODDS, K. S. The Seed Hairs of <i>Gossypium</i> . With Plate IV	361
JANAKI-AMMAL, E. K., see DARLINGTON, C. D.	
LAL, B. N. Plant-Injection Methods for the Diagnosis of Mineral Deficiencies in Tobacco and Soya Bean. With Plate III and eight Figures in the Text	283
MANTON, I. Chromosome Length at the Early Meiotic Prophases in <i>Osmunda</i> . With Plates I and II, and forty-nine Figures in the Text	155
MASON, T. G., and PHILLIS, E. Studies on the Partition of the Mineral Elements in the Cotton Plant. V. An Adsorption Theory of Nitrogen Regulation. With one Figure in the Text	335
— The Effect of Ringing and of Transpiration on Mineral Uptake: A Reply to Criticism. With one Figure in the Text	345
— see also PHILLIS, E.	
MILTHORPE, F. L. Fibre Development of Flax in Relation to Water Supply and Light Intensity. With eight Figures in the Text	31
PHILLIS, E., and MASON, T. G. Studies on Foliar Hydration in the Cotton Plant. VI. A Gel Theory of Cell Water Relations. With ten Figures in the Text	297
— The Effect of Extreme Desiccation on the Viability of Cotton Seed. With four Figures in the Text	353
— see also MASON, T. G.	
STEPHENS, S. G., see HUTCHINSON, J. B.	

- SWAMY, B. G. L., and THIRUMALACHAR, M. J. Homologies of the Embryo Sac of Angiosperms. With one Figure in the Text 179
- THIRUMALACHAR, M. J., see SWAMY, B. G. L.
- WARDLAW, C. W. Experimental and Analytical Studies of Pteridophytes.
 V. Stelar Morphology: The Development of the Vascular System. With twenty-eight Figures in the Text 217
 VI. Stelar Morphology: The Occurrence of Reduced and Discontinuous Vascular Systems in the Rhizome of *Onoclea sensibilis*. With Plates V and VI, and thirty-three Figures in the Text 383

Studies in the Comparative Morphology of the Algae

IV. Algae and Archegoniate Plants¹

BY

F. E. FRITSCH

(*Department of Botany, Queen Mary College, University of London*)

With twenty-two Figures in the Text

THE discovery of the existence of relatively unspecialized land-plants, the Psilophytales, during Silurian and Devonian times has served to bring out clearly the essential differences between the varied assemblage of plants grouped as Algae and those which for the sake of brevity may be spoken of as Archegoniatae. These differences are the possession of a cuticularized surface layer interrupted by stomata, the presence of vascular tissue of a simple type, and the cuticularized wind-borne spores. Even some of these features are not entirely peculiar to archegoniate plants (cf. below). Whatever place may be given to the Psilophytales in phylogenetic speculations, it is clear that at a relatively early era in the vegetation of the world there existed a diversity of leafless plants (*Rhynia*, *Hornea*, *Cooksonia*, Lang, 1937, p. 249; *Hicklingia*, Kidston and Lang, 1923) that might with equal justification be termed vascular thallophytes (cf. Arber, 1921, p. 47). Similar forms (*Psilophyton*, *Asteroxylon*), bearing more or less numerous small leaves, occurred in the same horizons. Plants of these simple types, and probably more or less closely allied to one another, have been found in fossil deposits as far apart as Scotland, western Germany, Canada, and Victoria, Australia (Lang and Cookson, 1930); and there is reason to believe that, even though perhaps restricted to special habitats, a Psilophytalean vegetation was in early Palaeozoic epochs world-wide in its distribution. That is to say, there must have flourished over a relatively long period of time plants that were removed from Algae only by the few special attributes mentioned above.

'In the case of some other Early Devonian plants, that are, however, very imperfectly known, we may with fuller knowledge have to recognize such combinations of characters as would break down any sharp distinction between the Algae and the simplest Pteridophytes' (Kidston and Lang, 1921, p. 843). It is within the realms of probability that a more intensive study of older Palaeozoic remains—at present investigated only in comparatively few localities—may yet reveal types of plants transitional between Algae and the Psilophytales or other Pteridophyta, and that it will disclose stages in the

¹ This is in part a restatement of the theory advanced nearly 30 years ago (Fritsch, 1916), but supported by the extensive data which have been provided by the researches of the intervening period.

evolution of land-forms. The 'fossil Algae' so far recorded from the older Palaeozoic strata show none of these stages and, although there can be little doubt that the waters of these periods were inhabited by an abundance of Algae of diverse kinds, it is only in very rare instances that any satisfactory systematic assignation can be made of the remains that have been discovered. Nor have they in general disclosed any marked variety of morphological construction or afforded evidence of progressive evolutionary changes.

In endeavouring to reconstruct the stages in the origin of archegoniate plants the various evolutionary tendencies displayed by present-day Algae should be taken into consideration. It is in fact possible, on the basis of comparative study of existing Algae, to formulate with some degree of probability the essential nature of the preliminary steps that led to the evolution of simple vascular plants comparable to the Psilophytales.

RECONSTRUCTION OF THE EARLY STAGES IN THE EVOLUTION OF ARCHEGONIATE PLANTS

In the shallow stretches around the margins of early Palaeozoic waters numerous types of Algae will have flourished, many of them probably derived from autotrophic flagellate ancestors, although there is no reason to believe that all the bottom-living forms that grew there had been derived from such a source. The Blue-green Algae in particular, which have probably been in existence from earliest times, afford no evidence of derivation from a previous flagellate stock and may well have originated from coccoid forms which were motionless from the first (Fritsch, 1929, p. 133). The same may be true of the Rhodophyceae. The origin of filamentous types from flagellate and coccoid stages, as illustrated by the ontogeny of present-day forms in nearly all algal classes, has often been discussed (Fritsch, 1929, p. 110; 1935, p. 17) and requires no special consideration here.

Emphasis must, however, be laid on the probable early appearance of heterotrichous types. It has been shown in the first article of this series (Fritsch, 1942) that this special habit has been attained in all the major algal classes and that it plays a very significant role in the organization of certain members of Chlorophyceae, Phaeophyceae, Rhodophyceae, and Myxophyceae (Fritsch, 1942*b*). It is clearly a stage of elaboration that has been reached wherever evolution of the plant-body has progressed beyond a certain level. The primary development of a prostrate system composed of creeping threads, capable of spreading over considerable areas of the surface of rock or mud, is a morphological elaboration eminently qualified for purposes of colonization in shallow water, since it affords at the same time a secure basis for attachment and a system well suited for photosynthesis. This may well have been a first step in the direction of evolutionary advance (Fritsch, 1942, p. 401; cf. also Meyer, 1910, p. 308), although the majority of such prostrate types among present-day Algae would seem to be reduced forms. Purely prostrate (*Ulvella*) or crust-forming (*Heribaudiella*, *Hildenbrandia*) heterotrichous Algae are in fact very successful colonizers of rocks

in streams (Fritsch, 1929a; Butcher, 1932), which in degree of movement of the medium present conditions perhaps analogous to those around the margins of Palaeozoic waters.

The outgrowth of erect filaments no doubt ensued early, and constituted a means of reaching farther towards the source of light and perhaps also into a region of the water better supplied with the necessary gases. We should thus have arrived at the first stage, the heterotrichous filament, postulated in the evolution of a land-plant from an algal ancestry. It should be pointed out that the sequence of events suggested is that which is actually followed in the individual development of the vast majority of filamentous heterotrichous Algae.

At this point reference may be made to a peculiar common characteristic of aquatic heterotrichous filamentous Algae, found alike in Chlorophyceae, Phaeophyceae, and Rhodophyceae, viz. the production of hairs at the tips of the diverse branches. The nature of these hairs varies in different groups and their degree of development is subject to considerable variation, even in one and the same species. There is evidence that dyes enter these hairs with special facility and the latter have also been regarded as having a respiratory significance (cf. Oltmanns, 1923, p. 390; Wille, 1897, p. 37; Rosenvinge, 1911, p. 214). They may well constitute a mechanism of some importance in the early evolution of aquatic heterotrichous types. It is noticeable that they are lacking in most of the terrestrial Trentepohliaceae among the heterotrichous Chlorophyceae.

In the less specialized Brown (Ectocarpales) and Red Algae (Nemalionales) the early stages of development are almost invariably of the nature of an heterotrichous filament, and the mature thallus originates from the further elaboration of one or more of the erect threads (Fritsch, 1942, p. 401). This elaboration may ensue in different ways, and there is no reason to doubt that such diverse methods were followed also in the further evolution of the heterotrichous filament when first it arose in Palaeozoic times. The fossil Prototaxites (Nematophyton), for example, is probably a multiaxial type¹ (cf. Kräussel and Weyland, 1934; Lang, 1926; 1937, p. 259; Seward, 1898, p. 192), whatever its exact affinities. Only one of the methods of thallus construction seen in present-day Algae is, however, of interest to us here, namely the parenchymatous one that is characteristic of the polystichous Ectocarpales. Such an elaboration of the upright system of the primary heterotrichous filament was no doubt a second step in the evolution of the land-plant. To afford a definite picture we can visualize stages such as are seen in the early development of a Scytosiphon or Stictyosiphon² (cf. also Figs. 1, 2).

It has already been stated that the origin of the parenchymatous type of organization 'marked a most significant evolutionary advance, since in it lay

¹ The reference of this genus to Laminariales (Kräussel and Weyland, 1934; Pia, 1927, p. 95), which are parenchymatous forms, is quite unjustified.

² Cf. also Goebel's (1915, fig. 478) drawings of young sporophytes of *Catharinea undulata*, although these lack a prostrate system.

the germ for the development of a plant-body of almost unlimited size' (Fritsch, 1943, p. 64). Indeed all the larger and highly specialized Phaeophyceae (Laminariales, Fucales)¹ are based on this type of construction. But, even in an alga like *Dictyosiphon foeniculaceus*, the minute heterotrichous

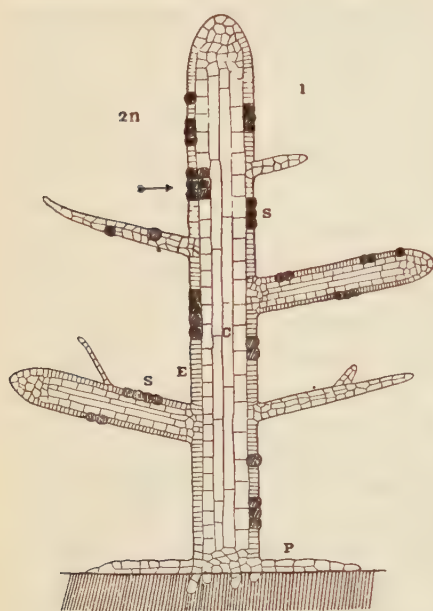


FIG. 1.

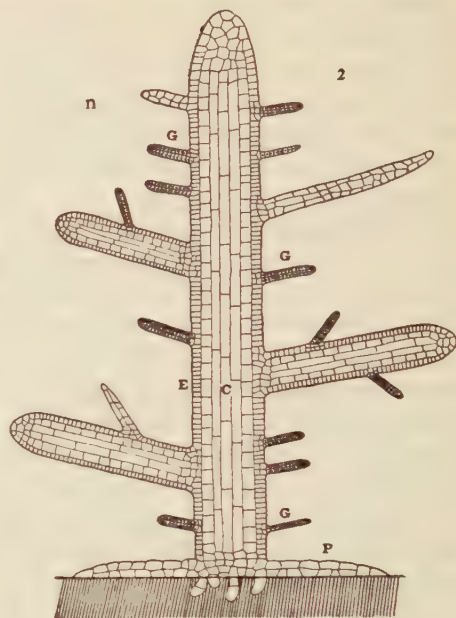


FIG. 2.

FIGS. 1 and 2. Representation in longitudinal section of the two isomorphic generations of an early type of hypothetical land-plant, still possessing diffuse growth. C, central conducting cells; E, erect system; G, gametangia; P, prostrate system; S, sporangia. At the place indicated by the arrow hypodermal cells are also acting as sporangia. Substratum shaded.

plantlets, which are parallel to the permanently minute gametophytes and which initiate the sporophytic stage (Sauvageau, 1929), exemplify the capacity for development of the erect branches of the primary heterotrichous filament into a thallus that may reach a length of 60 cm.

It seems probable that the parenchymatous strands of the algal ancestors early acquired apical growth. This is a feature of the prostrate system and of prostrate types among Chaetophorales, although in the majority of instances the erect threads exhibit diffuse growth. Vischer (1933, p. 58) has, however, described a genus *Caespitella*—allied to *Stigeoclonium*—which is stated to show apical growth, and the same is recorded for the erect threads of *Trentepohlia* (Brand, 1902; West and Hood, 1911). The replacement of intercalary by apical growth is probable in diverse lines of descent among Phaeophyceae

¹ It is apposite to point out in this connexion that the mature structure of *Fucus* and its immediate allies is specialized as compared with the majority of Fucales. The principal axes of such genera as *Halidrys*, *Cystoseira*, and *Sargassum* have a typical parenchymatous structure without appreciable separation of the cells by gelatinization of the walls and may contain relatively few hyphae.

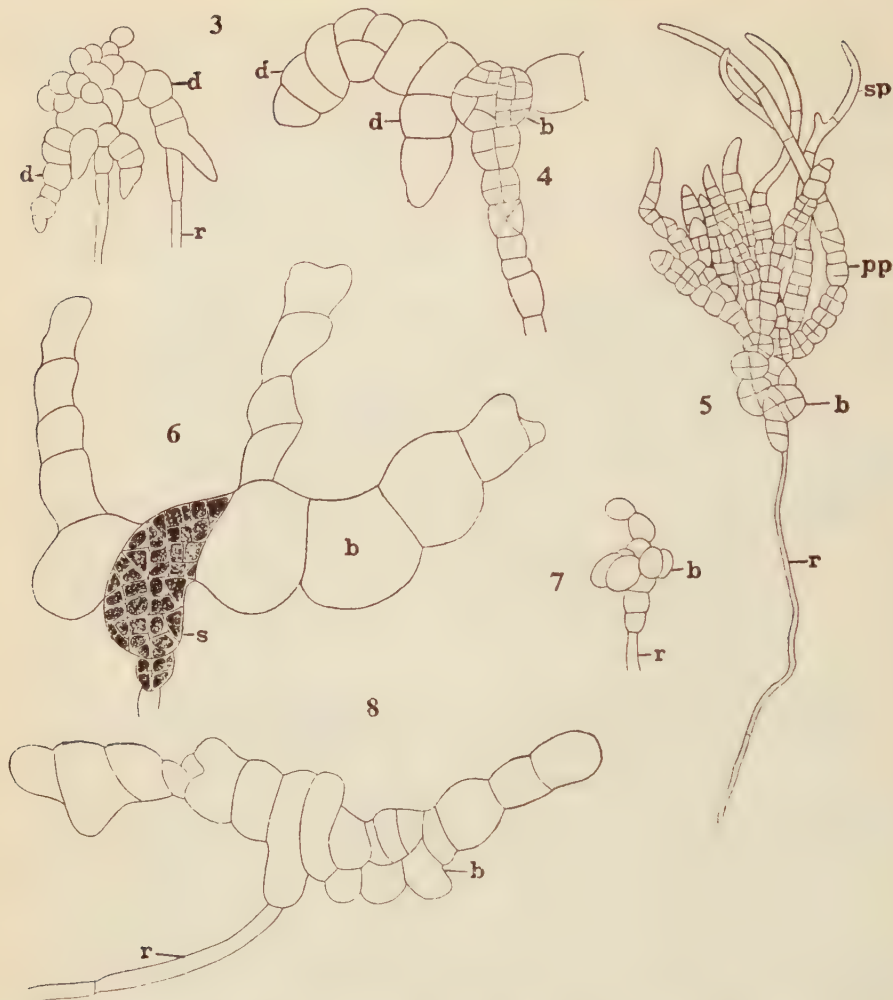
(cf. Fritsch, 1943, p. 67) and something of the same kind probably occurred in the parenchymatous erect strands of early land-plants. In this connexion attention may be drawn to the frequent occurrence of an intercalary meristem during the development of the sporogonia of Bryophyta (cf. Goebel, 1915, p. 535), a feature which is very pronounced in *Anthoceros* (cf. also the prothalli of certain species of *Lycopodium*). Dichotomous branching, which is unknown in any simple filamentous alga, must have followed in certain evolutionary lines on the inception of apical growth in the parenchymatous system (cf. Figs. 9, 10).

In Chlorophyceae, Phaeophyceae, and Rhodophyceae alike, many of the more specialized members (*Draparnaldia*, *Draparnaldiopsis*, and *Frittschiella* (Iyengar, 1932) among Chlorophyceae; *Sporochnales*, *Desmarestiales*, *Laminariales*, &c., among Phaeophyceae; *Ceramiales* among Florideae) lack the early heterotrichous stages seen in the less specialized forms (Fritsch, 1942, p. 400). A primary prostrate system is no longer formed and growth is erect from the first. This is evidently a definite evolutionary trend, and it is justifiable to suppose that it was followed also during the evolution of land-plants, although it is probable that heterotrichy was abandoned at different stages in different lines of descent. No evidence of the occurrence of this habit is to be found in any fossil plant or in the embryology of the sporophytes of Bryophyta or Pteridophyta.

It is, however, tempting to suppose that the heterotrichous habit still persisted in the early land-plants that were settling on damp mud round the margins of Palaeozoic waters, because of the facilities it afforded for rapid establishment and secure anchorage. Its disappearance possibly coincided with the development of a capacity for the lower parts of the erect axes to burrow into the mud and form a supplementary attachment system (cf. Figs. 9, 10). The terrestrial *Frittschiella* (Fig. 5) is of interest in this connexion. This genus of *Chaetophoraceae* was first found on the moist silt of drying rain-water pools (Iyengar, 1932), but has since been reported on various types of relatively dry soils in northern India (Randhawa, 1939; Singh, 1941). The upper cells of the short erect axis of *Frittschiella* develop by division in various planes into a secondary prostrate system (Figs. 5, 7, *b*) composed of a number of clusters of cells,¹ which are apparently sometimes situated beneath the surface of the soil. Moreover, there is a tendency for some of these clusters to grow out laterally and in a slightly downward direction into the soil (Figs. 3, 4, *d*). A similar development from the basal parts of an erect parenchymatous axis (cf. Figs. 1, 2) might result in the formation of underground growths serving for the anchorage of the former.

The primary projecting system of *Frittschiella* (Fig. 5, *pp*) that arises from the clusters (*b*) shows occasional longitudinal division, i.e. a parenchymatous tendency, and it is not unreasonable to suppose that a like tendency might develop in the threads that grow downwards from the basal clusters of cells.

¹ Judging by some of Singh's figures (1941, Figs. 10 and 11) this prostrate system may sometimes have a more definitely filamentous character (cf. Figs. 6, 8).



FIGS. 3-8. *Frittschiella tuberosa* Iyeng. 3, 4, development of downward-growing branches (*d*) from the cells of the prostrate system. 5, habit of a small mature plant. 6, prostrate system, one cell of which (*s*) is forming zoospores. 7, early stage of development. 8, filamentous prostrate system, with rhizoid. *b*, clusters of cells forming the secondary prostrate system; *d*, downwardly growing branches from same; *pp*, primary and *sp*, secondary projecting systems; *r*, rhizoids; *s*, zoospore-producing cell. (6 and 8 after Singh; the rest after Iyengar.)

In short *Frittschiella*, albeit in a somewhat specialized form, illustrates the existence of potentialities for a further elaboration in a Chaetophoraceous type in the direction above postulated for an early algal transmigrant (cf. also Bower, 1935, p. 498; Singh, 1941, p. 181). Attention may also be drawn to *Rhizothallus* (Dangeard, 1931), in which the prostrate system ramifies to some depth in the substratum.

There is no reason to suppose that the early inhabitants of the mud around the edges of Palaeozoic waters were anything but diminutive plants; in fact,

as compared with their brethren that remained beneath the water they may even have undergone dwarfing, such as is usually observed when aquatic plants colonize exposed mud. The more highly developed terrestrial Algae (*Fritschiella*, *Trentepohlia*, *Cladophorella*) are generally of low stature as compared with many of their aquatic allies. The *Trentepohliaceae*, especially those of the Tropics, show that, in a moderately humid environment, relatively simple heterotrichous types can maintain themselves successfully and even colonize very varied substrata (Fritsch, 1907, p. 242; van Oye, 1923). The same can be said of *Fritschiella* (cf. above). Given a capacity to develop under subaerial conditions, there is no reason at all against minute forms of this kind becoming progressively elaborated into more and more massive parenchymatous types. If this can happen in the sea, it might equally well happen on land.

The initiation of the next step, the differentiation of vascular tissue which perhaps took place at first only in the overground parts, is obscure. It should, however, be recalled that in parenchymatous *Phaeophyceae* pronounced elongation of the internal cells is the rule and, in a maturing *Chorda*, is accompanied by the differentiation of the specialized elements known as 'trumpet-hyphae'. That a simple parenchymatous body is capable of almost unlimited external and internal differentiation is illustrated by the *Laminariales* and *Fucales*; in certain of the former this leads to the development of elaborate sieve-tubes, strikingly parallel to those of higher plants. Scalariform thickenings on the walls of the medullary cells of *Sargassum* have been described and figured by Hansteen (1892, p. 342).

It is not unreasonable to suppose that the first parenchymatous terrestrial types possessed the same extraordinary capacity for elaboration, albeit that took place in a different direction to that followed in *Phaeophyceae*. There are clearly some factors militating against the production of lignified xylem-tissue in submerged plants, as shown by the majority of hydrophytic *Phanerogams*. It is not possible, in the present state of our knowledge, to say whether any marked elaboration had already taken place among aquatic Algae when simple parenchymatous types first became established on land, but the acquisition of lignified vascular tissue may be supposed to have followed upon the adoption of a terrestrial mode of life.

In view of the necessity of restricting water-loss from the exposed regions of the early colonizers of the land, the development of a protective mechanism must have been of immediate importance. A considerable number of terrestrial Green Algae (Fritsch, 1922), as well as moss protonema (Bristol, 1919), exhibit a great capacity for withstanding periods of drought, but so far as is known this entails adoption of a state of practical dormancy by the cells. Such mechanisms, whatever be their nature in detail, would scarcely suit the needs of an actively growing land-form. The development of a superficial cuticle, restricting transpiration, if combined with the differentiation of water-conducting tissue, would provide a sufficient protection against all but severe drought and would leave the cells in an active condition, capable of division

and of further elaboration. It is therefore not without interest that in *Cladophorella* (Fritsch, 1944) the surface layer of the membrane of the erect-growing threads shows all the essential features of a cuticle. Its discovery removes one further point of contrast between Algae and archegoniate land-plants. The essential corollary to the appearance of a cuticle, the creation of apertures admitting of gaseous exchange, must have ensued rapidly, but no data are available as to the manner of evolution of stomata.

Before proceeding farther the stages in the development of a land-plant arrived at above may be summarized. These are:

1. Production of a creeping prostrate system.
2. Development of erect branches, giving a heterotrichous filament.
3. Elaboration of one or more of the erect branches of such a filament, by division in various planes, into a parenchymatous growth.
4. Differentiation in the upgrowth between a peripheral photosynthetic system and a central system of elongate cells, probably playing some role in conduction (cf. Figs. 1, 2).
5. Development of a conducting system by the differentiation of phloem and xylem.
6. Secretion of a surface cuticle and accompanying differentiation of stomata.

Stages 1-4 are paralleled among existing *Phaeophyceae* (cf. also *Bangiales*), while 1 and 2 are those which comparative study shows to have been followed in all the major classes of Algae. The marked contrast between the mode of origin of land-plants just indicated and that assumed by Church (1919) may be emphasized. The first land-plants are supposed to have possessed a very simple structure and the diverse resemblances between the larger Algae and terrestrial plants upon which Church laid so much stress are regarded as nothing else than an outcome of inherent tendencies implicit in the parenchymatous type of organization. These tendencies resulted in the simple ancestors of land-plants developing to some extent along lines parallel to those followed by marine Algae.

THE IMMEDIATE AFFINITIES OF EARLY VASCULAR PLANTS

Most of the algal classes are distinguished by the possession of accessory photosynthetic pigments, with which are associated modifications of the metabolic processes as expressed by diversity in the food-reserves accumulated (fat, leucosin, mannitol, laminarin, Floridean starch, paramylon, &c.) and differences in the chemical composition of the cell-walls, although so far there is complete lack of knowledge as to the details of this relation. Among the classes with a pronounced development of cellular as opposed to flagellate and coccoid types, the *Chlorophyceae* alone possess chloroplast pigments and a photosynthetic mechanism comparable to those of higher plants. Moreover, cellulose is a frequent, though by no means invariable, constituent of the cell-wall.

By contrast to the two great marine classes the heterotrichous development among Chlorophyceae (Chaetophorales) is distinguished by the fact that it comprises only filamentous and relatively simple (in part perhaps primitive) prostrate types. The Chaetophorales do not in fact pass beyond the level illustrated by Ectocarpaceae among Phaeophyceae, Erythrotrichieae among Bangiales, and Acrochaetiaceae and Batrachospermaceae among Florideae. Yet the extensive range of forms exhibited by the order Siphonales testifies to a capacity for vegetative advance, although along individual lines, which falls little short in certain respects of that attained among some of the more highly differentiated Brown and Red Algae. Moreover, among Siphonales, elaboration takes place in the direction of uniaxial (Dasycladaceae) and multiaxial (Codiaceae) developments, parallel with that seen in many septate Brown and Red Algae, while Caulerpa may be ranged at the side of the parenchymatous development in other classes. It is also relevant that the Dasycladaceae have been traced back to the earliest Palaeozoic deposits (Pia, 1923, 1927), since when they have undergone progressive specialization (see also Fritsch, 1935, p. 397).

If one bears these facts in mind, it is difficult to deny to the septate, non-siphonaceous Chlorophyceae a capacity for vegetative advance far beyond that shown by the present-day forms and, with the record of the Dasycladaceae before us, to suspect that this capacity existed already in the early eras of their existence. The Siphonales are almost entirely marine, and there is no evidence that they ever underwent any marked development in fresh waters. It might, therefore, be argued that the great elaboration exhibited by them, as well as by the Phaeophyceae and Rhodophyceae, was only possible in a marine environment and that the relatively low stage of vegetative development attained by other Chlorophyceae is a result of their having evolved as a freshwater group (Church, 1919, p. 8). This argument, however, contains a fallacy.

There is no adequate reason for regarding the Chlorophyceae as a predominantly freshwater group. Certain orders, like the Oedogoniales, Conjugales, and Charales, are indeed confined to fresh, including in the case of the last brackish, waters, but all the others contain a more or less appreciable number of marine representatives. The relatively few records of marine flagellate (Volvocales) and coccoid (Chlorococcales) Green Algae (cf. Printz, 1927, pp. 40, 86) are probably but a result of imperfect knowledge, and there is some reason to believe that they may not be uncommon in littoral communities which, apart from their larger seaweeds, have so far been very inadequately studied. In the remaining orders of the Green Algae, distinguished by the writer (Fritsch, 1935), there is a marked marine development, as evidenced by the many Ulotrichales (including Ulvaceae), Cladophorales, and Chaetophorales found in the sea (cf. also the Siphonales).

The Chaetophorales are represented by *Pilinia* (Acroblaste, Reinsch, 1879; Newton, 1931, p. 60; Collins, 1908), in which the vegetative body is a heterotrichous filament resembling that of some species of *Stigeoclonium*, as

well as by an appreciable number of prostrate types (*Phaeophila*, *Acrochaete*, *Pringsheimia*, *Ulvella*, &c.). It is pertinent to ask why, in view of the existence of such relatively simple heterotrichous Chaetophorales in the sea, on the same level of development as that displayed by diverse members of *Phaeophyceae* and *Rhodophyceae*, they have undergone no further elaboration comparable to that which has occurred abundantly in the two great marine classes. The presence of such forms in the sea warrants the supposition that, had evolution continued there, a development of types analogous to those of the brown and red seaweeds would have taken place. I am of the opinion that such elaboration did follow in the shallow waters of prehistoric seas, but that the resulting forms invaded the land and became adapted to a terrestrial existence as the first land-plants (cf. Fritsch, 1916).

It may be granted that, perhaps as a result of paucity of mineral nutriment, the survivors of the Chaetophorales that play an appreciable role as lithophytes in rivers and lakes have failed to show this further elaboration, but that is no reason to assume that members of this group could not have found conditions congenial to their development under terrestrial conditions. Perhaps there is something in the metabolism of the Green Alga, as opposed to that of other holophytic algal classes, that favoured elaboration under terrestrial rather than under marine conditions. It appears quite unnecessary to postulate a mythical group of green forms that colonized the land. The Chaetophorales contain all the germs requisite for the development of more complex types and the parenchymatous *Phaeophyceae* in particular show what could be attained starting from such beginnings.

It is apposite in this connexion to take note of the marked capacity for terrestrial adaptation displayed by the *Chlorophyceae* as a whole (cf. Fritsch, 1921, p. 168) and quite especially evident among Chaetophorales. With the description of *Cladophorella* (Fritsch, 1944) the last of the orders of *Chlorophyceae* has yielded up its right to be regarded as an entirely aquatic group. Among the predominantly aquatic Chaetophorales there are a number of striking terrestrial genera, ranging from *Iwanoffia* (Iwanoff, 1900; Pascher, 1905), and alga structurally similar to and closely allied to *Stigeoclonium*, through such a specialized form as *Frittschiella* (cf. also the little known *Oliveria*, Nayal, 1935, p. 206), to reduced types like *Pleurastrum* (Chodat, 1894; Vischer, 1933, p. 16; Fritsch and John, 1942, p. 383) which is not uncommon in the soil. Even more than these, however, the *Trentepohlieae* afford a token of the marked capacity of members of Chaetophorales for a subaerial existence and lend added support to the view that it was heterotrichous Green Algae resembling those included in the present-day Chaetophorales that gave origin to early types of land-plants. It is of course quite improbable that any of the diverse terrestrial Chaetophorales living at the present day are relicts of the actual transmigrants. They are rather to be regarded as evidence of the capacity of the heterotrichous Green Alga to survive under subaerial, in part even under rather extreme (cf. Singh, 1941, p. 181), conditions. They represent attempts to colonize land which have not

resulted in further elaboration and, for all we know, may be of comparatively recent origin.

In the foregoing matter I have made no reference to the debated question of the composition of the medium in early Palaeozoic waters (Knopf, 1931, p. 65), since it is immaterial to my main theme. If, as has been held by some, the majority of the seas in those days were far more dilute than those of the present, then the further development of the heterotrichous stage took place in the increasingly saline seas in the case of Phaeophyceae and Rhodophyceae, and on the land in the case of Chlorophyceae. Neither of the marine classes shows any great aptitude for a terrestrial existence, although the seaweeds of the littoral region are able to survive temporary exposure and the Fucales provide us with more extreme examples of terrestrial accommodation in the shape of the diverse growth-forms, most of them greatly stunted, that flourish on salt-marshes (Baker and Bohling, 1916).

REPRODUCTION AND LIFE-CYCLE OF PRIMITIVE LAND-PLANTS

There are no adequate grounds for supposing that the early land-plants had necessarily attained to an oogamous sexual process. The known oogamous filamentous types among present-day Chlorophyceae are for the most part outliers whose immediate affinities are difficult to determine, and the prevalent sexual reproduction throughout the class is an isogamous or anisogamous one. Among Phaeophyceae oogamy is in general associated with types of thallus-construction that, on the basis of comparative considerations, must be regarded as advanced, and the relatively simple heterotrichous parenchymatous forms among Ectocarpales all show an isogamous sexual reproduction. The bulk of the Chaetophorales are isogamous, and this is true also of many of the more highly specialized genera (*Draparnaldia*, *Draparnaldiopsis*, *Frittschiella*, *Trentepohlia*), but *Aphanochaete* (Huber, 1894) and *Chaetonema* (Meyer, 1930) are oogamous, although the female cell is liberated prior to fertilization and, in *Aphanochaete*, is a quadriflagellate swarmer which however soon ceases to move. *Coleochaete*, the only member of Chaetophorales with retention of the ovum and affording clear indications of specialization in its sexual reproduction, illustrates the marked isolation that characterizes most of the oogamous types among Green Algae. It is noteworthy that oogamy is not found among Chaetophorales in any form showing specialization in the erect system.

The present-day oogamous Green Algae, however, exemplify the existence of a tendency towards oogamy in practically every line of development and, moreover, for the most part display the two outstanding features of archegoniate oogamy, viz. the presence of but a single ovum and its retention after fertilization. It is therefore not improbable that the early parenchymatous land-types arising from a Chaetophoraceous stock were isogamous (cf. Kylin, 1933, p. 94), although the capacity for the development of oogamy in the manner typical for Chlorophyceae in general will have been inherent in them. Since growth was probably gregarious, the meeting of gamete with gamete on

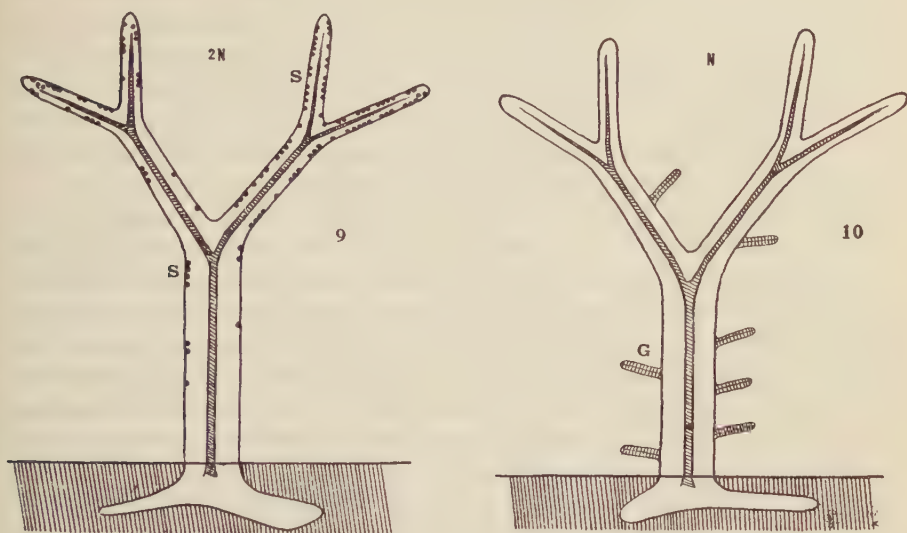
the wet rock or mud surface will have presented no difficulties and germination of the zygote was no doubt immediate (cf. below).

In the present state of our knowledge the mode of origin of the complex sex organs characteristic of archegoniate plants remains conjectural. Various authorities have, however, supported the view that the two kinds of sex organs have diverged from a common ancestral type of multicellular gametangium (cf. Bower, 1935, p. 515; Davis, 1903, p. 481; Goebel, 1902; Holferty, 1904, p. 120; Lyon, 1904, p. 288; Schenck, 1908, p. 4). Without expressing agreement over all points of detail, it must be conceded that both archegonia and antheridia suggest derivation from a multicellular structure in which every cell produced a single functional gamete, although this is more patent in the case of the male than of the female organs. Such a structure is afforded by the plurilocular sporangium of the less specialized Phaeophyceae, and various writers (Lyon, 1904; Schenck, 1908) have considered the possibility of the derivation of archegoniate sex organs from a gametangium of this type.

There is no adequate reason to assume that a multiseptate sex organ was evolved only in the Phaeophyceae. The cells of Chaetophoraceae commonly produce only a single swarmer apiece, and such cells frequently occur in short rows (cf. Fritsch, 1935, p. 257); in *Draparnaldia* and *Draparnaldiopsis* (Singh, 1942, p. 265) entire laterals may be used up in swarmer-formation. Given a tendency for longitudinal septation, such as probably existed in early parenchymatous types, the origin of branches composed of several rows of swarmer-producing cells, i.e. a plurilocular sporangium, is not difficult to envisage (cf. Figs. 2, 10). It should be noted in this connexion that in *Draparnaldiopsis* (Singh, 1942, p. 265) swarmer-production is preceded by transverse division of vegetative cells (cf. also Fig. 6, s), while according to Meyer (1930, p. 150) the antheridia of *Chaetonema* are produced by transverse and longitudinal (cf. his Figs. 5 and 6) division of vegetative cells, although each compartment forms a number of male cells. The tendency here exhibited for the occurrence of septation in connexion with gamete-production shows that no great stretch of imagination is necessary to visualize the production of a structure, similar to the plurilocular sporangium of Phaeophyceae, in the Chaetophoraceous ancestry of land-plants. In the further elaboration of such a structure to produce the sex organs of Archegoniatae more or less appreciable sterilization must have occurred, but no evidence is available as to the course of evolution that was followed.

The preferential development of the lowest cell of the axile series in the archegonium may have resulted from its immediate proximity to sources of food-supply and have led to changes that bring about the ultimate degeneration of the other cells (canal cells). Since, however, a trend towards oogamy has evidently been the rule in the evolution of Algae, other causes—at present quite unknown—may well have cooperated. The establishment of oogamy in the multiseptate organ that preceded the archegonium was no doubt associated with retention of the ovum and subsequent nursing during embryo-development, and this may have resulted in profound modifications in the

latter (Lang, 1909). The example of the Phaeophyceae shows, however, that divergent development of the two generations can take place also in series in which the ovum is liberated from the female organ prior to fertilization. We may perhaps see in the suspensor met with in diverse groups of Pteridophyta a survival of the rhizoidal cell cut off for purposes of attachment in the free-living embryo, as in so many present-day Algae (cf. p. 18).



FIGS. 9 and 10. Representation in longitudinal section of the two isomorphic generations of an early type of hypothetical land-plant, with apical growth, dichotomous branching, and a developing underground system. G, gametangia; S, sporangia (in great part still superficial). Substratum shaded.

As regards the asexual organs, attention may be concentrated on the primitive type afforded by the Psilophytales, from which the sporangium-bearing organs of diverse other Pteridophyta can be more or less clearly derived. As was first emphasized by Schenck (1908, p. 31), comparison must be instituted between the spore mother-cell producing a tetrad of spores and the sporangia of the diploid phases of Algae in which spores are formed as a result of meiosis (cf. also Kidston and Lang, 1921, p. 851); such comparison is particularly striking with the tetrasporangia of Dictyotales and the diplobiontic Florideae. The spore-forming region of a Rhynia or Hornea can be interpreted as a number of tetrasporangia embedded in the apical tissues of the overground axes (cf. Fig. 11). Nothing of this kind is known in Chaetophorales or in any of the truly parenchymatous seaweeds; the sporangia of the latter are commonly immersed in the thallus, though not completely surrounded by sterile tissue. The red seaweeds, in which such enclosure occurs and which are drawn into the comparison by Kidston and Lang (1920, p. 622; cf. also Arber, 1921, p. 49), are pseudo-parenchymatous forms, the thallus of which is composed of a coalescent branch-system.

It seems probable that the first terrestrial Thallophyta, if they possessed differentiated sporangia at all, produced these peripherally (cf. Figs. 1, 9) and in direct contact with the medium, as in most present-day Algae. The change that resulted in the sporangia being produced in the interior of the parenchymatous thallus must have been a very momentous one, a very profound adaptation to subaerial existence. No adequate explanation can be provided as to its mode of occurrence. One can only suppose that in early parenchymatous types sporangia were embedded in the peripheral tissues (as in many present-day Ectocarpales, e.g. *Punctaria*, *Dictyosiphon*, *Coilodesme*), that other cells of the internal tissues (cf. the arrow in Fig. 1) gradually took on the role of spore-development,¹ and that finally this faculty was lost by the peripheral tissues themselves (Fig. 11). Such changes may have been associated with, or have followed upon, the secretion of a surface cuticle.

It was shown in the second article of this series (Fritsch, 1942a, p. 557) that there is considerable evidence for the view that, when a morphological and cytological phase-alternation first became established among Algae, it was of the isomorphic type. With reference to the reproductive processes discussed above, it is noteworthy that the fully substantiated instances of isomorphic alternation among Green Algae (*Ulvaceae*, *Cladophoraceae*, *Anadyomene*, *Microdictyon*) all comprise forms with an essentially isogamous sexual process. This is also true of two advanced genera of *Chaetophoraceae*, viz. *Draparnaldiopsis* (Singh, 1942) and *Fritschiella* (Singh, 1941). Although a cytological proof is still lacking for these two instances, the fact that plants produce either zoospores or gametes is suggestive of the existence of an isomorphic life-cycle.

There are also some indications that such a life-cycle may occur in certain of the terrestrial *Trentepohlias* (cf. Fritsch, 1935, p. 278). There is no doubt that the organs interpreted as gametangia can produce isogametes capable of sexual fusion (Karsten, 1891, p. 53; Wille, 1887); such organs are, however, often borne on the same plants as produce the asexual sporangia and in such instances their swimmers germinate without fusion (Meyer, 1936). There is a suggestion here of the existence of two kinds of organs which are identical in appearance, the one a sporangium, the other a gametangium, but it is useless to speculate farther upon this point until the life-cycles of various species of *Trentepohlia* have been worked out in full. Indications of the existence of isomorphic alternation in other *Chaetophorales* are afforded by Juller's observations on *Stigeoclonium subspinosum* (cf. Fritsch, 1942a, p. 538), by certain differences between the sexual and asexual individuals of *Pringsheimia* (Reinke, 1889; Fritsch, 1935, p. 264), and by the fact that Meyer (1930) did not observe zoospores in his sexually reproducing plants of *Chaetonema*.

The capacity to adopt an isomorphic life-cycle, which thus probably exists in certain members of present-day *Chaetophorales*, may well have obtained also in the early heterotrichous forms that colonized the land. Whether they

¹ An analogous centripetal transference of spore-forming tissue is assumed by Goebel (1915, p. 739) to take place in the *Anthocerotales*.

attained to such a life-cycle before or after transmigration must remain an open question, but the probability is in favour of such a life-cycle having already been acquired because, among present-day Chlorophyceae, it appears largely at least to be associated with vegetative advance, such as would be exemplified by the development of a parenchymatous structure in the erect system (cf. also Kylin, 1933, p. 94). There is no reason to suppose that the zygotes of Palaeozoic Algae normally entered upon a resting stage, as is characteristic of the majority of existing freshwater Chlorophyceae. That is likely to have come as an adaptation to existence in smaller bodies of water liable to periodic drying up. All isomorphic forms show immediate germination of the zygote without a resting period, and this is also true of all marine Algae. The only change necessary, therefore, in the haploid ancestry for the establishment of an isomorphic life-cycle is postponement of the reduction divisions (Fritsch, 1942a, p. 536), such as has evidently occurred in diverse lines of descent.

Consideration of the Ectocarpales justifies the view that the heteromorphic type of life-cycle, which is abundantly represented there, has originated from the isomorphic type by divergent development of the two phases. No clear instance is known among present-day Chaetophorales (cf., however, *Stigeoclonium subspinosum*). It is possible that *Urospora* (Jorde, 1933) and *Derbesia* (Kornmann, 1938) may afford examples in other series of Chlorophyceae; both, however, require further elucidation. There is no reason to doubt that a capacity for divergent development of the two phases did exist among the early heterotrichous terrestrial representatives of Chaetophorales. The advantages that would accrue from a gradual restriction of sex organs on the haploid phase to the basal parts of the erect system (cf. Fig. 10) or to an elaborating prostrate system whereby the moisture requisite for fertilization would remain available, and of the restriction of spore-producing organs on the diploid phase to an elaborating erect system affording facilities for ready wind-dispersal (cf. Fig. 9), are so patent that one is tempted to suspect some operation of the new environment in bringing about such divergent development. It is even plausible to conjecture that the effects of desiccation on those sex organs that were located on the upper parts of the erect system of the haploid phase—organs probably ill adapted to resist such conditions—may have led to their atrophy. On the other hand, the wind-borne spores of the diploid phase may early have acquired that intolerance of free moisture during their maturation that distinguishes them in the majority of present-day land-plants so that only those situated on the erect system could survive.

Summarizing the content of this section it may be suggested that the Chaetophoralean ancestry of land-plants early exhibited a parenchymatous development of the erect and in certain evolutionary lines also of the prostrate system (Figs. 1, 2), that it possessed an isomorphic life-cycle with a haploid phase reproducing isogamously and bearing multicellular and probably multiseriate gametangia forming a single gamete in each cell (Figs. 2, 10) and a diploid phase bearing superficial (although perhaps immersed) sporangia,

which may or may not have already reached the stage of producing only four spores (Figs. 1, 9). Further progression resulted in gradual differentiation of the gametangia, complete enclosure of the sporangia (spore mother-cells) within sterile tissue (Fig. 11), and divergent development of the two phases. It is impossible to say at what stage differentiation of vascular tissue, cuticle, and stomata ensued, but these forward steps must clearly have taken place early during transmigration if the race was to survive.

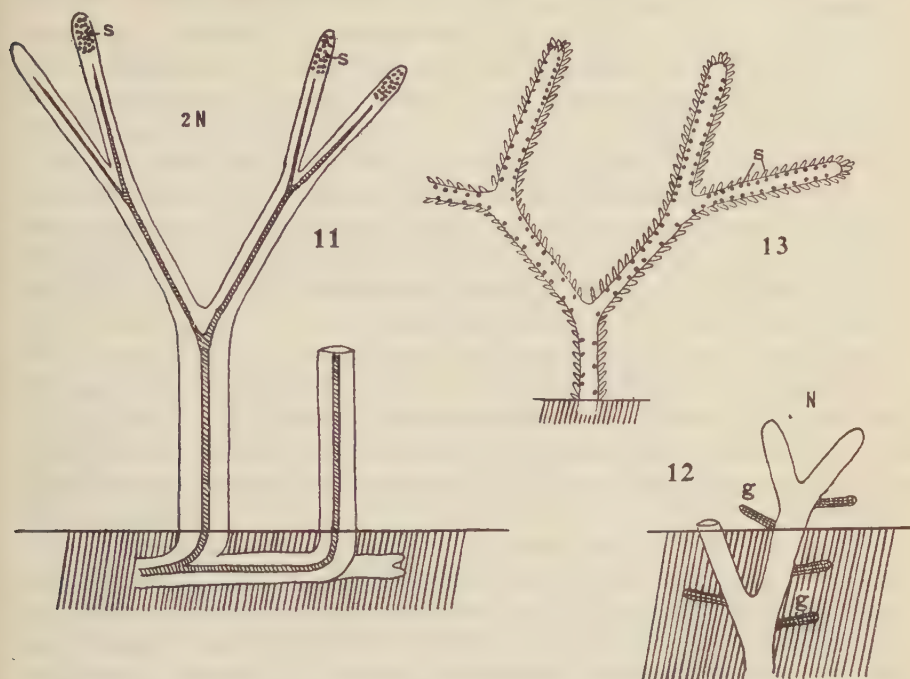
CONSIDERATION OF ARCHEGONIATE PLANTS FROM THE STANDPOINT OF A HETEROTRICHOUS GREEN ANCESTRY

(1) *Pteridophyta*

There are no marked difficulties in linking up the evolutionary progress of land-plants outlined in the preceding pages, on the basis of a comparative study of existing Algae, with the condition exemplified by the sporophyte of a Rhynia or a Hornea among Psilophytales. The interpretation of other Pteridophyta in terms of the morphological status attained by these fossil forms has been pursued by others (cf. Kidston and Lang, 1921; Bower, 1935) more qualified than I to undertake such a task, and no purpose would be served by discussing these matters in detail here. Although the Psilophytales have undoubtedly helped to clarify our views on Pteridophyte morphology, there has perhaps been too marked a tendency to assume that all primitive land-plants were of the known Psilophytalean type (cf. e.g. Zimmermann, 1930, pp. 28, 104, 127, 174) and to ignore the possibility that there may have been other kinds of primitive land-plants, so far undiscovered or imperfectly known, that would admit of easier interpretations in certain directions. In view of the wide diversity of parenchymatous forms that have evolved from the heterotrichous stage among Phaeophyceae, there is every reason to suspect that the heterotrichous transmigrant Green Algae may have developed in diverse directions, culminating in different series of Pteridophyta (cf. Arber, 1921; Bower, 1935, p. 517). This does not necessarily imply a polyphyletic origin, since the various types may have arisen from a common ancestral filamentous form. The probability must further be taken into consideration that the divergent development of the two generations was initiated at different stages and took place in different ways in the various evolutionary series. Finally the location of the sporangium-bearing zones may have varied.

I have no hesitation in accepting the view that the Psilotales were derived from Psilophytales resembling Asteroxylon or Psilophyton. The origin of the small leaves of the latter as enations is now supported by a considerable body of comparative data (cf. e.g. Lang, 1931, p. 439; Bower, 1935, p. 553), in which the Palaeozoic fossil flora has furnished many of the key-stones. The interpretation of the fertile systems of Psilotales as condensed lateral branch-systems, analogous to those of Asteroxylon, is plausible. The prothalli, as well as the young subterranean embryos, are radially constructed, and various

authorities (Lawson, 1917; Holloway, 1939, p. 333) have commented on the marked similarity between the gametophytes and young sporophytes in this group; in *Psilotum* both propagate vegetatively by gemmae of a practically identical type. The facts suggest that, in the ancestry of this series, the prostrate system was lost at an early stage and that the two generations



FIGS. 11-13. 11, Representation in longitudinal section of the diploid generation of a later type of land-plant with immersed sporangia (*S*) and approximating to a *Rhynia*. 12, Hypothetical gametophyte generation of the same. *G*, gametangia. 13, Representation of the diploid stage of a hypothetical polycopod type, with embedded lateral fertile tracts. Substratum shaded.

diverged comparatively late, after an underground system had been established to which the gametangia were relegated (cf. Fig. 12). The gametophytes are to be interpreted as corresponding to the primary underground growth of the sporophyte and as having lost the capacity for emergence overground. The occurrence of a vascular strand in certain prothalli of *Psilotum* (Holloway, 1939, p. 322), although unique so far as gametophytes are concerned, is not surprising on this interpretation. If embryos and gametophytes of *Psilophytales* are ever discovered, it would not be astonishing to find that the latter were of the same general nature as those of *Psilotales*; the gametophytes may even have been less reduced (cf. e.g. Fig. 12).

The two generations of *Lycopodiaceae* probably originated in the same way as those of *Psilotales*, although the ancestral type may well have been of a somewhat different pattern. Those members of the family, that on

comparative grounds are regarded as primitive, have erect-growing radially organized gametophytes and sporophytes. The universal occurrence in the embryology of a suspensor, which, with Bower (1935, p. 526), I incline to regard as comparable to the basal attachment-organs (rhizoids) of Algae, perhaps indicates a relatively late adoption of retention of the ovum. The fertile system of the sporophyte, with single sporangia orientated medianly with respect to the leaves (enations), is not easy of interpretation by reference either to Rhynia or to Asteroxylon. It is probable that in the ancestral type the groups of spore mother-cells (tetrasporangia) were immersed in a lateral rather than in a terminal position in the parenchymatous thallus (Fig. 13) and that their emergence as superficial outgrowths may have been a secondary development and related to the production of the leaves. What is known of the early stages of development of the sporangia of *Lycopodium* is not antagonistic to such an interpretation. It may be noted that in the gametophytes immersion of the antheridia (as compared with the condition in Psilotales) is the rule and that the archegonia likewise are deeply sunk.

A number of early Palaeozoic fossils are known, showing appreciable resemblance to the true Psilophytales in certain details of construction, but possessing single laterally disposed sporangia. *Zosterophyllum* (Lang, 1927; Lang and Cookson, 1930, p. 149) was leafless, but in *Baragwanathia* (Lang and Cookson, 1935) the relatively thick axes were rather densely clothed with long simple lax leaves, with large reniform sporangia in certain zones; 'the indications are that they were situated adaxially in relation to the leaf-bases, though this is not proved' (Lang and Cookson, 1935, p. 435). The authors of *Baragwanathia* point (p. 442) to the resemblance to *Lycopodium* and express the opinion that the position of the sporangia 'would formally separate the plant from the Psilophytales as originally defined'. The two genera just mentioned may well be members of an alliance from which the modern *Lycopodium* evolved. It seems possible that this alliance and the Psilotales may be the descendants of two related ancestral stocks, possessing many features in common, but differing in the location of the fertile tracts in the diploid phase (cf. Figs. 11 and 13). A different ancestry is also suggested by the divergent nature of the spermatozoids in the two groups.

The other Lycopodiales are so highly specialized that they afford few criteria to indicate their origins. The herbaceous heterosporous line illustrated by *Selaginella*, like that represented by *Lycopodium*, appears to go back to the Palaeozoic and shows certain points of contact with the Lycopodiaceae which may imply a remote common origin. Of the arboreal series, represented by the Palaeozoic *Lepidodendraceae* and *Sigillariaceae*, with *Isoetes* as a probable present-day survivor, it is scarcely possible to say even that. All Lycopodiales may, however, be supposed to have originated from an ancestry with laterally disposed fertile systems.

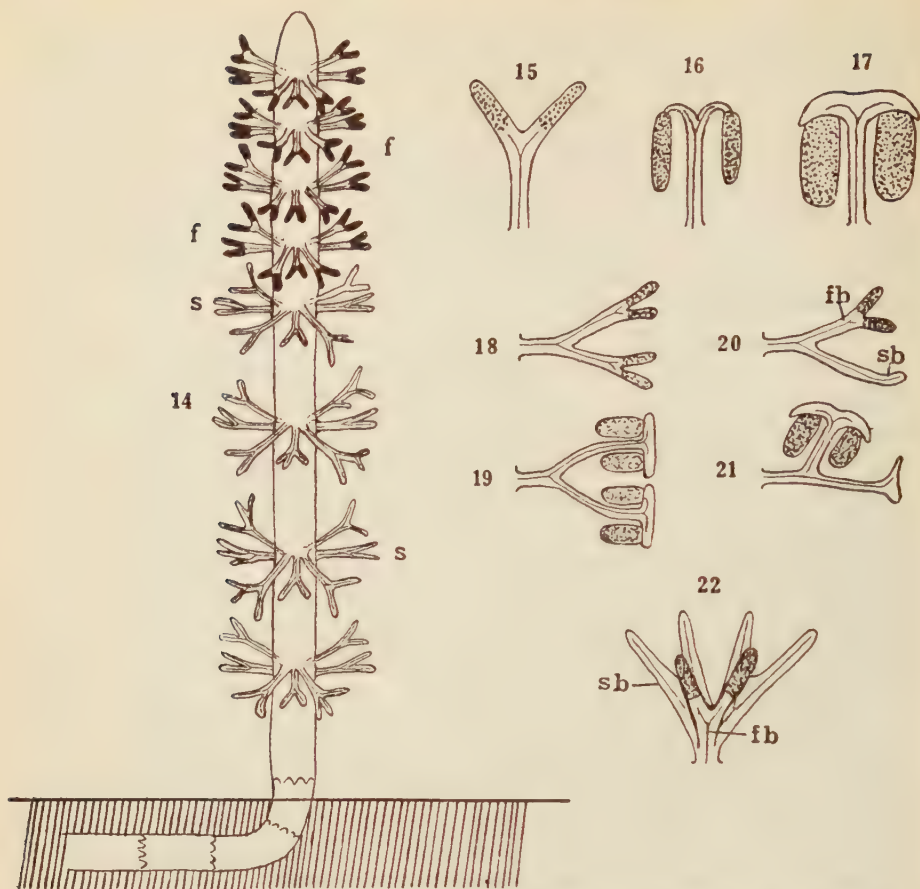
The remaining members of the former group Lycopsida, namely the Equisetales, Sphenophyllales, and certain imperfectly known Devonian fossil types (*Pseudobornia*, *Hyeria*, *Calamophyton*), differ markedly in their

morphological construction from the Pteridophyta hitherto considered. The disposition of the leaves in whorls separated by elongate internodes is common to all true Equisetales and Sphenophyllales and, apart from certain similarities in the fertile systems, justifies classification in a common group the Articulatales (Scott's and Arber's Sphenopsida) and the assumption that its two main series represent divergent developments from a common ancestral stock (cf. Scott, 1923, p. 409). The marked differences in anatomical structure have been bridged by the demonstration of the protostelic condition of seedling *Equisetum* (Barratt, 1920) and the discovery of centripetal xylem in *Calamites* (*Protocalamites*) *pettycurensis*.

The whorled microphyllous leaves found in the younger members of both series of Articulatales are often regarded as enations, comparable to those of Psilophytales and Lycopodiales (cf., however, Arber, 1921, p. 73), but there are manifestly very great difficulties in the way of accepting this. The older representatives of both series—the more ancient species of Sphenophyllum, and Asterocalamites (Archaeocalamites) among Equisetales—possess leaves which are repeatedly forked, and a still more striking instance is met with in Pseudobornia where the forks of the leaf are pinnately compound. Comparison of the older with the younger species of Sphenophyllum justifies the view that the leaves have undergone simplification combined with webbing (Hirmer, 1927, p. 348; Bower, 1935, p. 149), and the same may also be true of the Equisetales.

Many present-day Algae exhibit a more or less clearly defined whorled branching (Draparnaldiopsis; Charales; Cladostephus, &c., among Sphacelariales; Arthrocladia among Desmarestiales; Batrachospermum and many other simple Florideae). There is every reason to suspect that forms possessing such whorled branching appeared also among the early ancestry of land-plants, and it is credible that types with the general organization of Psilophytales, but bearing whorled lateral appendages, may yet come to light. Arber (1921, p. 74) has already advanced a similar view; he directs attention to certain obscure early Palaeozoic fossils and thinks it conceivable 'that they may have been among the ancestors of the Sphenopsida', although too dubious to give any marked weight to the suggestion. The appendages of the ancestral algal type, though subsidiary to the main axes, may well have shown at first the same dichotomous branching as the axes themselves (cf. Fig. 14); this is actually so in Hyenia from the Upper Devonian and to a lesser extent in Calamophyton from the same horizon. Flattening, combined with webbing and some reduction in size, would afford all the types of leaves known among Articulatales.

The view just propounded that the foliar appendages of Articulatales are cladode-leaves, which have originated in a differentiating whorled parenchymatous thalloid type, simplifies the interpretation of the wide range of strobili met with in this group. Certain Equisetales (Asterocalamites, Phyllothea, Equisetites, Equisetum), which may well represent a special line of development, possess strobili in which whorls of sporangium-bearing appendages



FIGS. 14-22. 14, Representation of the diploid stage of a hypothetical early member of Articulatales. *f*, fertile and, *s*, sterile whorls. The sterile appendages are assumed to have already undergone some flattening. Substratum shaded. 15, fertile appendage of 14 enlarged. 16, *Calamophyton primaevum* Kräusel and Weyland, fertile appendage. 17, sporangiophore of *Asterocalamites*. 18, fertile appendage, seen from the side, of a hypothetical type with dorso-ventral branching (see p. 21). 19, fertile appendage of *Sphenophyllostachys fertile* Scott seen from the side. 20, side view and, 22, view from above of a fertile appendage branching dorso-ventrally in which the upper limb only is fertile (see p. 21). 21, hypothetical type transitional between 20 and a *Calamostachys*. *fb*, fertile and, *sb*, sterile lobes.

(sporangia) are grouped in close proximity, although at intervals sometimes (apparently normally so in *Phyllothea*) interrupted by a whorl of sterile leaves. In other Equisetales (*Calamostachys*, *Palaeostachya*, *Cingularia*), as well as in *Cheirostrobos* and all the more typical species of *Sphenophyllostachys* (*S. fertile*, Fig. 19; *S. Dawsoni*; *S. Roemerii*), there is a dorso-ventral division of the fertile appendages in the vertical plane, the dorsal and ventral lobes being either in direct juxtaposition or, as in *Calamostachys*, spatially separated. Such dorso-ventral division is obviously the more widespread condition.

In the *Asterocalamites-Equisetum* series the whorls of sporangiophores may be regarded as derived from branches of the ancestral thallus, equivalent to those from which the leaves originated, though modified in relation to their special function (cf. Figs. 14, 15). A condition possibly illustrating an intermediate stage in their elaboration is seen in *Calamophyton* (Fig. 16), where the fertile appendages, though different in form, fork into two lobes like the foliage leaves, each lobe bearing a pendent sporangium. Should Lacey's observations (1943) on the four-lobed character of the head of the sporangiophore in *Calamostachys* prove to apply also to the outwardly similar sporangiophores of *Asterocalamites*, then the resemblance to *Calamophyton* would be considerable. The occasional whorls of sterile leaves seen in the strobili of the series of forms under discussion are readily comprehensible, if leaves and sporangiophores are both primarily derived from the whorled laterals of the ancestral type.

In the main series of *Equisetales* and *Sphenophyllales* it must be supposed that the fertile whorls underwent branching, not only in the horizontal, but also in the vertical plane, both sets of branches being fertile and retaining the original cylindrical form (Figs. 18, 19, *Sphenophyllostachys fertile*, cf. also *Cingularia Cantrilli*) or one set (the fertile sporangiophores) retaining it, while the other underwent flattening and foliar development to form the subtending bracts (Figs. 20-2). Such an interpretation does not necessitate the hypothesis of an invasion of the fertile by the sterile whorls in *Equisetales* and *Sphenophyllales* (Bower, 1935, pp. 596, 598) and also explains the intimate vascular relations between sterile and fertile members of the strobili in *Cheirostrobos*, *Sphenophyllostachys Dawsoni*, *Calamostachys*, and *Palaeostachya* which are difficult to understand on an 'invasion' hypothesis. Bower actually adopts (1935, p. 598) an explanation like that put forward here to account for *S. fertile*, although he does not extend it to the other instances. Hirmer (1927, p. 469 et seq.) has attempted to correlate all the diverse types of strobili found in *Articulatales* by recognizing in each a so-called 'sporophyll-unit' and, although I am insufficiently acquainted with some of the forms involved to appraise his scheme in detail, the sporophyll-unit may well in general represent a single (simple or variously branched) member of the fertile system. Zimmermann (1930, p. 178) discusses this interpretation and dismisses it on what appear to me to be false premises.

Although nothing strictly comparable to the dorso-ventral branching of the fertile appendages, assumed to have occurred in the evolution of the main series of *Articulatales*, is known among *Algae*, attention may be drawn to the branching of the whorled laterals of *Cladostephus verticillatus* in more than one plane. Another, though more remote analogy, is seen in *Sargassum* where the lateral systems branch in the vertical plane, the lowest member developing into a leafy structure, while others remain cylindrical and become fertile (Oltmanns, 1889, p. 55). This instance is instructive as illustrating how different parts of a thalloid branch-system can give rise respectively to foliar organs and to fertile receptacles.

Embryos and prothalli are known only in *Equisetum*. The embryonic axis grows erect so that the sporophytes afford no indications of a prostrate system; the horizontal underground rhizome is secondary. The prothalli, on the other hand, are at first prostrate dorsiventral growths, though later developing lobes (specially pronounced in the female gametophytes), which bend up towards the light. We may well have the two parts of the original heterotrichous plant represented in the two generations of *Equisetum* (cf. Goebel, 1918, Fig. 933), just as the two so sharply contrasted phases of *Cutleria* almost certainly originated from the divergent development of the two parts of such a system (Fritsch, 1942, p. 409); cf. also the condition met with in *Laminariales* and *Desmarestiales*. In the prothalli of *E. debile* (Kashyap, 1914) the leafy outgrowths arising from the upper surface of the prostrate growth may perhaps be the equivalents of an erect system.

The interpretation of the fronds of Filicales as cladode-leaves is now generally accepted, and it is not proposed to consider the diverse data that can be brought forward in its support. Bower (1923, p. 81) has given *in extenso* the evidence for the view that the fern-frond is derived from an originally dichotomous system which, in the more specialized forms, has passed over to monopodial branching. A somewhat parallel series can be found among Fucales (*Fucus*—*Ascophyllum*—*Seirococcus*—*Halidrys*). No traces of heterotrichy are recognizable in the embryos of Filicales and the frequent dorsiventral symmetry of the mature shoot is secondary. On the other hand, in all the leptosporangiate ferns, the gametophyte is a prostrate dorsiventral structure, though more variable in pattern than is usually assumed (Goebel, 1918, p. 948). As in *Equisetum*, the two generations appear to be the equivalents of the two parts of the ancestral heterotrichous system. Where a fern-plant is produced apogamously (cf. the figure on p. 492 of Bower, 1935), the two parts of the system appear in what is tantamount to a single plant. Such instances are analogous to Sauvageau's observations on the vegetative origin of new *Cutleria*-plants from the *Aglaozonia*-stage of *C. monoica* (cf. Fritsch, 1942, p. 409), although here the erect gametophytic stage arises from a prostrate growth, homologous with the sporophyte, though probably haploid.

In interpreting the fern gametophyte as an elaborated prostrate system of a heterotrichous ancestry, one is confronted with the difficulty presented by the ventral position of the sex organs. It must be supposed that the latter were primarily borne on the upper surface of the prostrate system, and there is no clue as to the way in which transference to the under surface was accomplished. It is possible that the prothallus was at first a creeping cylindrical (and perhaps branched) structure bearing sex organs along the sides as well as on the upper surface (cf. the archegonium-bearing laterals of a *Trichomanes*-prothallus; Goebel, 1918, p. 155) and that, with the development of wings, the reproductive organs became relegated to the lower surface. It is known that, when prothalli are illuminated equally on both surfaces, archegonia may be formed also on the upper side (Bower, 1923, p. 278; cf. also Leitgeb, 1879) and the mode of development of the prothallus, and

especially of the prothalli of diverse Schizaeaceae (Bauke, 1878), suggests the possibility that the wings are more recent developments than the midrib (cushion). It may well be that the filamentous prothalli of Schizaea and of diverse Hymenophyllaceae are a nearer approach to the ancestral type, although whether a comparatively recent reversion or a primitive condition lingering on in special habitats (Goebel, 1918, p. 958) it is impossible to say.

There is reason to believe that the diploid phase of the thalloid ancestry of ferns comprised plants that rapidly attained to an appreciable stature and considerable ramification. Branching was probably entirely or prevalently dichotomous and the fertile tracts were terminal (cf. *Stauropteris*), although probably early tending to become aggregated (as in *Cladoxylon scoparium*, *Etapteris*, *Corynepteris*). The Coenopteridaceae show us that differentiation of branch-systems as photosynthesizing organs may in part have preceded the development of foliar expanses. The ancestral type will have had considerable resemblances to that leading on to Psilophytales, but it may be doubted whether it was identical.

It must remain an open question whether all ferns emanated from a common ancestral type. The Ophioglossaceae differ from all other living Filicales in their radially constructed, erect-growing gametophytes and, like the Psilotales and Lycopodiales, have lost the prostrate system in both generations; the *Botrychium*-prothallus is probably derivative (Goebel, 1918, p. 951). Whilst there is nothing intrinsically against the possibility of development of the gametophyte in different directions from a single ancestral source, the many other peculiarities of the Ophioglossaceae suggest caution in assuming an ancestry in common with that of the leptosporangiate ferns. The occurrence of a suspensor in various Ophioglossaceae is also suggestive of a somewhat different evolutionary history. Such provisos apply equally to the Marattiaceae, where, however, the gametophyte is dorsiventral, though more robust than the usual leptosporangiate type. The origin or origins of the eusporangiate ferns must therefore, on present evidence, remain uncertain; maybe they are the outcome of a separate evolution along one or more lines from a thallophyte stock related to, but not identical with, that from which the bulk of the ferns arose.

The assessment of the various groups of Pteridophyta on the basis of the assumed heterotrichous algal ancestry thus leads to the view that, from the primitive type, there may have originated a considerable number of evolutionary series, differing in their mode of ramification, in the disposition of the sporangial tracts, and in the mode of development of the gametophytic stage from the isomorphic progenitor. In the Psilophytales-Psilotales and in the Lycopodiaceae (perhaps in all Lycopodiales) both generations lost the prostrate system and may well have been much alike for some time. In the Equisetales and the bulk of the Filicales (Leptosporangiales), however, the prostrate system was perpetuated in the gametophyte, as in so many present-day Phaeophyceae. The diversity of types assumed to have evolved from the heterotrichous ancestry, although for the most part unsupported by direct

fact, finds a certain measure of parallel in the many different evolutionary series, emanating from a similar ancestry, that can be traced among Phaeophyceae (Fritsch, 1943, p. 83).

(2) *Bryophyta*

The Musci appear to be the only Archegoniatae in which both parts of the heterotrichous ancestor have persisted in a single phase. The protonema is the equivalent of the prostrate system, the moss-plant that arises from it the equivalent of the erect system. The relations of the two parts are quite similar to those seen in many of the more advanced Ectocarpales and in the Nemalionales. In the Hepaticae, on the other hand, it is seemingly only the prostrate system, though in many series in a highly elaborated form, that persists in the gametophyte. It may be doubted whether the relatively rudimentary protonemal stages, met with in many of the thalloid Hepaticae, are the exact equivalents of the protonema of a moss. The early stages of germination show resemblances to those seen in certain of the more advanced Ectocarpales (cf. Kylin, 1933, Fig. 31, A-D), as well as in other Phaeophyceae (Desmarestiales, Laminariales), where the rounded cell formed from the zoospore (embryospore) puts out a tubular prolongation into which most or all of the contents ordinarily pass, the cell cut off at the tip of the prolongation dividing to produce the prostrate system of the gametophyte. Both in the Algae mentioned, as well as in the liverworts under discussion (Meyer, 1910, p. 309; Goebel, 1915, p. 760), the indirect germination of the spore is probably a device serving to carry the developing gametophyte into regions of more favourable illumination. Certain foliose liverworts show more complex juvenile stages, but the relations between these and the mature gametophytes do not indicate a derivation from the two systems of the heterotrichous ancestry; it seems probable that they are biological adaptations conditioned by the environment.

The sporophyte of Bryophyta is unique in its unbranched character and in its permanent, more or less marked dependence on the gametophyte. As in the higher Brown and Red Algae and the sporophytes of Pteridophyta, it has lost all traces of a prostrate system. In the usual distal production of spores there are resemblances to Psilophytales. The Florideae, in the special instances afforded by such forms as *Liagora tetrasporifera* and *Phyllophora Brodiaei*, illustrate two different ways in which the dependent sporophyte of Bryophyta might have arisen (cf. Fritsch, 1942a, p. 556). There are no positive data to indicate which course was followed, and any endeavour to arrive at a definite conclusion must be markedly influenced by the way in which the facts of comparative morphology of the sporogonia are interpreted. If *Riccia* and its allies are regarded as primitive, the Bryophyte sporogonium is probably a new elaboration of a post-sexual phase. If, however, *Anthoceros* be regarded as a relatively primitive type (Goebel, 1915, p. 739; Bower, 1935, p. 110), then there is increased probability that the two generations of Bryophyta were at one time heterotrichous and isomorphic and that the sporogonium is an

asexual generation that has secondarily become dependent on the gametophyte and, in relation to this, has undergone far-going modification. The resemblances between gametophyte and sporophyte in *Anthoceros*, which have been specially stressed by Goebel (1915, p. 545), lend appreciable support to the latter view. Other evidence is perhaps afforded by the occasional presence of rhizoids on the sporogonia of various Bryophyta (cf. Goebel, 1915, p. 550).

On the basis of the available evidence it seems probable that the Bryophyta have followed a blind evolutionary line of their own (cf. also Arber, 1921, p. 71). There is no great difficulty in linking up such a form as *Anthoceros* with the hypothetical ancestral type; the sporophyte having eliminated the prostrate and the gametophyte the erect system. If there was a single evolutionary line *via* an *Anthoceros*-like ancestor, it must have been distinct from those that gave rise to the various groups of Pteridophyta, although possibly related in some way to that leading to the Psilophytales-Psilotaes series. It was distinguished by the unbranched character of the erect system and the early adoption of a dependent habit on the part of the sporophyte. The very varied development of the prostrate system in the gametophytes, especially of Hepaticae, suggests that the Bryophyte line may soon have diverged in a number of different directions.

GENERAL CONCLUSIONS AND SUMMARY

The assumptions as to the mode of origin of land-plants put forward in this paper are based on the comparative morphology of existing Algae, certain special aspects of which have been dealt with in the preceding papers of this series. In view of its basic importance in the two great marine classes and its wide representation in all algal groups that have attained to a certain level of somatic differentiation, the heterotrichous filament is regarded as the probable starting-point for the evolution of land-forms. The prostrate system is excellently suited for the purposes of colonization of the surface of mud or rock in shallow marginal waters and may well have preceded the outgrowth of erect filaments. The next step was probably the development of a parenchymatous structure in the latter and in some groups also in the prostrate system by division of the cells in various planes, and this was accompanied by a differentiation between small-celled peripheral photosynthetic and long-celled central conducting (and perhaps mechanical) regions (cf. Figs. 1, 2). All these stages are paralleled by the ontogeny of the sporophyte of one of the parenchymatous Ectocarpales (cf. also Sphacelariales).

There is reason to assume that the erect-growing parenchymatous strands at first possessed intercalary growth, which sooner or later was replaced by apical growth; with the assumption of the latter dichotomous branching became possible (Figs. 9, 10). Invasion of the substratum by the basal parts of the erect system probably ensued as soon as soil began to be formed. The development of vascular tissue in the region of the medulla and the production of a surface cuticle would afford a plant suited to subaerial life and in general resembling a *Rhynia* or *Hornea*. There is nothing to indicate the

mode of origin of vascular tissue, but its differentiation marks no greater forward step than is displayed by many of the features of internal structure seen in Laminariales. It has recently been established that green terrestrial Algae can secrete a surface membrane approximating to a cuticle.

It is suggested that land-plants originating in the way above described were derived from forms closely resembling the Chaetophoraceae of the present-day. These forms are typically heterotrichous, show a tendency towards longitudinal division of the cells, include a number of more or less specialized terrestrial genera, and are also represented in the littoral region of the sea (*Pilinia*, *Pringsheimia*, &c.). They are parallel to the simple heterotrichous members of Brown and Red Algae, and the only adequate explanation for the absence of more specialized septate green forms in the sea is that such forms developed the equipment for a terrestrial, rather than a marine, existence.

Since progression in somatic development among Green Algae (incl. Chaetophoraceae) is commonly associated with the acquisition of an isomorphic life-cycle, there is reason to believe that such a life-cycle had already been attained in the transmigrant green alga, and since all Green Algae possessing such a life-cycle (cf. also Ectocarpaceae) are isogamous, it is probable that the transmigrant was at first isogamous. The frequent production of single swarmers from the cells, and the not uncommon utilization of whole laterals for this purpose, among Chaetophoraceae justifies the belief that, with the acquisition of parenchymatous construction, organs resembling the plurilocular sporangia of Phaeophyceae arose also in this line of descent (Figs. 2, 10). The derivation of archegonia and antheridia from such structures, though speculative, is not impossible. It is suggested that the sporangia (tetrasporangia?), at first superficial, gradually became embedded in the tissue of the sporophyte (Fig. 11), affording spore mother-cells as the basis of the future asexual reproductive organs.

The further development of the two generations, perhaps under the operation of the terrestrial environment, will have led to a divergence of sporophyte and gametophyte and the assumption of a heteromorphic life-cycle. The derivation of the latter from the isomorphic type has been demonstrated among Phaeophyceae. As in the two marine classes, the prostrate system was lost in the further evolution of the sporophyte, and sometimes also in that of the gametophyte. This is so in Psilotales and Lycopodiaceae (and perhaps all Lycopodiales), while in Equisetales, most Filicales, and in the Bryophyta the prostrate system is represented by the gametophyte (in mosses by the protonema). The mosses are the only living archegoniate plants that have retained both parts of the ancestral heterotrichous system in their gametophytes.

While a generalized heterotrichous filamentous type may have been common to all the series of evolving Archegoniatae, there is reason to believe that there was early divergence along a number of different lines. The line ancestral to the Psilotales probably resembled the Psilophytales (Fig. 11). That giving

rise to the Lycopodiales was probably distinguished by lateral, instead of terminal spore-forming regions (Fig. 13). That leading to the Articulatales (Equisetales, Sphenophyllales) is assumed to have possessed the whorled branching found in so many present-day Algae (Fig. 14). That resulting in the leptosporangiate Filicales will have resembled the Psilophytales, though probably early becoming richly branched in a dichotomous manner, while the mode of origin of the eusporangiate ferns remains doubtful. That affording the Bryophyta will have been distinguished by the unbranched character of the sporophyte which had terminally immersed spore-mother cells. The leaves, as well as the sporangiophores, of Articulatales are regarded as originating from branches of the whorled ancestral thallus (cf. Fig. 14). Although the evidence is not conclusive, the sporophyte of Bryophyta is probably derived from a former free-living generation (cf. *Phyllophora Brodiaei*).

With large areas of the earth's surface still practically unexplored so far as their marine and freshwater vegetation is concerned, it is not impossible that there may yet be found types of Green Algae, comparable to *Frittschiella* first described in 1932, that will furnish added support for, or call for modifications in, the views expressed in this paper. The marked degree of parallelism in evolution displayed by the major algal groups, however, justifies a belief that essentially the same type of progress obtained also in the early evolution of land-plants.

LITERATURE CITED

- ARBER, E. A. N., 1921: Devonian Floras. A Study of the Origin of Cormophyta. Cambridge.
- BAKER, S. M., and BOHLING, M. H., 1916: On the Brown Seaweeds of the Salt-marsh. II. Journ. Linn. Soc. London, Bot., xliii. 325.
- BARRATT, K., 1920: A Contribution to our Knowledge of the Vascular System of the Genus *Equisetum*. Ann. Bot., xxxiv. 201.
- BAUKE, H., 1878: Beiträge zur Keimungsgeschichte der Schizaeaceen. Jahrb. wiss. Bot., xi. 603.
- BOWER, F. O., 1923: The Ferns (Filicales). Vol. I. Cambridge.
- 1935: Primitive Land Plants. London.
- BRAND, F., 1902: Zur näheren Kenntnis der Algengattung *Trentepohlia* Mart. Beih. Bot. Centralbl., xii. 200.
- BRISTOL, B. M., 1919: On the Retention of Vitality by Algae from old stored Soils. New Phytol., xviii. 92.
- BUTCHER, R. W., 1932: Notes on New and Little-known Algae from the Beds of Rivers. Ibid., xxxi. 289.
- CHODAT, R., 1894: Matériaux pour servir à l'Histoire des Protococcoïdées. I. Bull. Herbier Boissier, ii. 585.
- CHURCH, A. H., 1919: Thalassiphyta and the Subaerial Transmigration. Oxford Bot. Mem., iii.
- COLLINS, F. S., 1908: The Genus *Pilinia*. Rhodora, x. 122.
- DANGEARD, P., 1931: Sur un Genre nouveau de *Trentepohliacées* récolté en Islande (*Rhizothallus* nov. gen.). Bull. Soc. Bot. France, lxxviii. 91.
- DAVIS, B. M., 1903: The Origin of the Archegonium. Ann. Bot., xvii. 477.
- FRITSCH, F. E., 1907: A General Consideration of the Subaerial and Freshwater Algal Flora of Ceylon. Proc. Roy. Soc. London, B, lxxix. 197.
- 1916: The Algal Ancestry of the Higher Plants. New Phytol., xv. 233.

- FRITSCH, F. E., 1921: Thalasssiophyta and the Algal Ancestry of the Higher Plants. *Ibid.*, xx. 165.
- 1922: The Moisture-relations of Terrestrial Algae. I. *Ann. Bot.*, xxxvi. 1.
- 1929: Evolutionary Sequence and Affinities among Protophyta. *Biol. Reviews*, Cambridge, iv. 103.
- 1929a: The Encrusting Algal Communities of Certain Fast-flowing Streams. *New Phytol.*, xxviii. 165.
- 1935: The Structure and Reproduction of the Algae. I. Cambridge.
- 1942: Studies in the Comparative Morphology of the Algae. I. Heterotrichy and Juvenile Stages. *Ann. Bot.*, N.S., vi. 397.
- 1942a: *Ibid.* II. The Algal Life-cycle. *Ibid.*, N.S., vi. 533.
- 1942b: The Interrelations and Classification of the Myxophyceae (Cyanophyceae). *New Phytol.*, xli. 134.
- 1943: Studies in the Comparative Morphology of the Algae. III. Evolutionary Tendencies and Affinities among Phaeophyceae. *Ann. Bot.*, N.S., vii. 63.
- 1944: *Cladophorella calcicola* nov. gen. et sp., a Terrestrial Member of Cladophorales. *Ibid.*, N.S., viii. 157.
- and JOHN, R. P., 1942: An Ecological and Taxonomic Study of the Algae of British Soils. II. *Ibid.*, N.S., vi. 371.
- GOEBEL, K., 1902: Ueber die Homologie in der Entwicklung männlicher und weiblicher Sexualorgane. *Flora*, xc. 279.
- 1915: Organographie der Pflanzen. 2nd edit. ii. 1. Bryophyten. Jena.
- 1918: Organographie der Pflanzen. 2nd edit. ii. 2. Pteridophyten. Jena.
- HANSTEEN, B., 1892: Studien zur Anatomie und Physiologie der Fucoideen. *Jahrb. wiss. Bot.*, xxiv. 317.
- HIRMER, M., 1927: Handbuch der Palaeobotanik. I. München and Berlin.
- HOLFERTY, G. M., 1904: The Archegonium of *Mnium cuspidatum*. *Bot. Gaz.*, xxxvii. 106.
- HOLLOWAY, J. E., 1939: The Gametophytes, Embryo, and Young Rhizome of *Psilotum triquetrum* Swartz. *Ann. Bot.*, N.S., iii. 313.
- HUBER, J., 1894: Sur l'Aphanochaete R. Br. et sa reproduction sexuée. *Bull. Soc. Bot. France*, xli. xciv.
- IWANOFF, L., 1900: Ueber neue Arten von Algen und Flagellaten, etc. *Bull. Soc. Imp. Nat. Moscou*, N.S., xiii. 423.
- IYENGAR, M. O. P., 1932: Fritschiella, a New Terrestrial Member of the Chaetophoraceae. *New Phytol.*, xxxi. 229.
- JORDE, I., 1933: Untersuchungen über den Lebenszyklus von Urospora Aresch. und Codiolum A. Braun. *Nyt Mag. Naturvidensk.*, lxxiii. 1.
- KARSTEN, G., 1891: Untersuchungen über die Familie der Chroolepideen. *Ann. Jard. Bot. Buitenzorg*, x. 1.
- KASHYAP, S. R., 1914: The Structure and Development of the Prothallus of *Equisetum debile* Roxb. *Ann. Bot.*, xxviii. 163.
- KIDSTON, R., and LANG, W. H., 1920: On Old Red Sandstone Plants showing Structure, from the Rhynie Chert Bed, Aberdeenshire. Part III. *Trans. Roy. Soc. Edinburgh*, lii. 643.
- — 1921: *Ibid.* Part IV. *Ibid.*, lii. 831.
- — 1923: Notes on Fossil Plants from the Old Red Sandstone of Scotland. I. *Hicklingia Edwardi*, K. and L. *Ibid.*, liii. 405.
- KNOPF, A., 1931: Physics of the Earth. IV. The Age of the Earth. *Bull. Nat. Res. Council, Nat. Acad. Sci. Washington, D.C.*
- KORNMANN, P., 1938: Zur Entwicklungsgeschichte von Derbesia und Halicystis. *Planta*, xxviii. 464.
- KRÄUSEL, R., and WEYLAND, H., 1934: Algen im deutschen Devon. *Palaeontogr.*, lxxix B, 131.
- KYLIN, H., 1933: Ueber die Entwicklungsgeschichte der Phaeophyceen. *Lunds Univers. Årsskr.*, N.F., xxix. 2, No. 7.
- LACEY, W. S., 1943: The Sporangiophore of Calamostachys. *New Phytol.*, xlii. 98.
- LANG, W. H., 1909: A Theory of Alternation of Generations in Archegoniate Plants based upon the Ontogeny. *Ibid.*, viii. 1.
- 1926: On the Identification of the Large 'Stems' in the Carnyllie Beds of the Lower Old Red Sandstone as Nematophyton. *Trans. Roy. Soc. Edinburgh*, liv. 792.

- LANG, W. H., 1927: Contributions to the Study of the Old Red Sandstone Flora of Scotland. VI. Ibid., lv. 443.
- 1931: On the Spines, Sporangia, and Spores of *Psilophyton princeps*, Dawson, shown in specimens from Gaspé. Phil. Trans. Roy. Soc. London, B, ccxix. 421.
- 1937: On the Plant-remains from the Downtonian of England and Wales. Ibid., B, ccxxvii. 245.
- and COOKSON, I. C., 1930: Some Fossil Plants of early Devonian Type from the Walhalla Series, Victoria, Australia. Ibid., B, ccxix. 133.
- 1935: On a Flora, including vascular Land Plants, associated with Monograptus, in Rocks of Silurian Age, from Victoria, Australia. Ibid., B, ccxxiv. 421.
- LAWSON, A. A., 1917: The Gametophyte Generation of the Psilotaceae. Trans. Roy. Soc. Edinburgh, lii. 93.
- LEITGEB, H., 1879: Ueber Bilateralität der Prothallien. Flora, xxxvii. 317.
- LYON, F., 1904: The Evolution of the Sex Organs of Plants. Bot. Gaz., xxxvii. 280.
- MEYER, A., 1910: Die Vorvegetation der Pteridophyten, der Gymnospermen, Angiospermen und Bryophyten. Eine Hypothese. Ber. Deutsch. Bot. Ges., xxviii. 303.
- MEYER, K. J., 1930: Ueber den Befruchtungsvorgang bei *Chaetonema irregulare* Nowak. Arch. Protistenk., lxxii. 147.
- 1936: Germination des zoospores et des gamètes chez le Trentepohlia Mart. Bull. Soc. Nat. Moscou, S. Biol., xlv. 96.
- NAYAL, A. A., 1935: Two New Members of the Chaetophorales from Egypt. Ann. Bot., xlix. 205.
- NEWTON, L., 1931: A Handbook of the British Seaweeds. London.
- OLTMANN, F., 1889: Beiträge zur Kenntnis der Fucaceen. Bibl. Bot., xiv.
- 1923: Morphologie und Biologie der Algen. 2nd edit. III. Jena.
- VAN OYE, P., 1923: Zur Biologie von Trentepohlia auf Java. Hedwigia, lxiv. 175.
- PASCHER, A., 1905: Zur Kenntnis der geschlechtlichen Fortpflanzung bei Stigeoclonium sp. Flora, xcv, Ergänzungsband, 95.
- PIA, J., 1923: Einige Ergebnisse neuerer Untersuchungen über die Geschichte der Siphoneae Verticillatae. Zeitschr. indukt. Abst. u. Vererb.-Lehre, xxx. 63.
- 1927: Thallophyta, in M. Hirmer, Handbuch der Palaeobotanik. München and Berlin, 31.
- PRINTZ, H., 1927: Chlorophyceae. Natürl. Pflanzenfamilien, 2nd edit., iii.
- RANDHAWA, M. S., 1939: A Note on Cyst Formation in *Frittschiella tuberosa* Iyengar. Arch. Protistenk., xcii. 131.
- REINKE, J., 1889: Atlas deutscher Meeresalgen. I. Berlin.
- REINSCH, P. F., 1879: Ein neues Genus der Chroolepideae. Bot. Zeit., xxxvii. 361.
- ROSENVINCE, L. K., 1911: Remarks on the hyaline unicellular Hairs of the Florideae. Biol. Arb. til. Eug. Warming, Kjöbenhavn, 203.
- SAUVAGEAU, C., 1929: Sur le Développement de quelques Phéosporées. Bull. Stat. Biol. Arcachon, xxvi. 253.
- SCHENCK, H., 1908: Ueber die Phylogenie der Archegoniaten und der Characeen. Engler Bot. Jahrb., xlii. 1.
- SCOTT, D. H., 1923: Studies in Fossil Botany. 3rd edit. Vol. ii.
- SEWARD, A. C., 1898: Fossil Plants for Students of Botany and Geology. I. Cambridge.
- SINGH, R. N., 1941: On some Phases in the Life History of the Terrestrial Alga, *Frittschiella tuberosa* Iyeng., and its Autecology. New Phytol., xl. 170.
- 1942: Reproduction in *Draparnaldiopsis indica* Bharadwaja. Ibid., xli. 262.
- VISCHER, W., 1933: Ueber einige kritische Gattungen und die Systematik der Chaetophorales. Beih. Bot. Centralbl., li. 1, 1.
- WEST, G. S., and HOOD, O. E., 1911: The Structure of the Cell-wall and the Apical Growth in the Genus Trentepohlia. New Phytol., x. 241.
- WILLE, N., 1887: Ueber die Schwärmzellen und deren Copulation bei Trentepohlia Mart. Jahrb. wiss. Bot., xviii. 426.
- 1897: Beiträge zur physiologischen Anatomie der Laminariaceen. Univers. Festkr. til H. Maj. Kong Oscar II, Regjæringsjubilaet.
- ZIMMERMANN, W., 1930: Die Phylogenie der Pflanzen. Jena.

Fibre Development of Flax in Relation to Water Supply and Light Intensity¹

BY

F. L. MILTHORPE

(*N.S.W. Department of Agriculture, Sydney*)

With eight Figures in the Text

IN order to understand the effect of environment on fibre production in flax, it is not sufficient to measure fibre yield at one stage only. The developmental anatomy of the plant must be studied; and for a complete understanding of the final effects as measured at maturity the fibre content of the plant throughout the growth period must be followed.

Although few studies have been made on the quantitative development of fibre in flax, there have been investigations of the structure and development of the fibre and of the fibre of mature stems. Anderson (1927) discussed the origin of the fibre cells and their development, showing that they originate in the meristem of the stem tip, the fibres being formed by successive depositions of cellulose within the primary walls. Anderson and Tammes (1907) both pointed out that at least some fibre cells increase in cross-sectional area during the whole life of the plant, following an early, rapid, and intensive increase in area and length immediately after their differentiation. Tammes stated also that from the cotyledons to the apex of the stem the area of fibre cells decreases and their length increases. Searle (1923) found an increase in the cross-sectional area both of stem and fibre after flowering, due to an increase in both the size and number of fibre cells. Recently Tiver (1942) has shown that secondary deposition of cellulose occurs first in the outer layer of cells of each bundle. Deposition then takes place progressively towards the inner boundary of each bundle. With low moisture supply, Tiver found that the inner cells failed to develop. An examination of one plant (Searle, 1928) showed that the fibre system consists of short unbranched bundles, which terminate at leaf-traces and occur only at the root end of the stem, and anastomosing bundles which run throughout the entire length of the stem. Since some of these bundles end at leaf-traces, the number of bundles found at any cross-section of the stem is approximately the same.

The experiment to be described was carried out to study the developmental anatomy of the flax stem, and of the fibre in particular, throughout growth and under four conditions of water supply and light intensity. A quantitative

¹ This work was carried out during tenure of the Walter and Eliza Hall Agricultural Research Fellowship at the University of Sydney.

[*Annals of Botany*, N.S. Vol. IX, No. 33, January, 1945.]

study of the flax plant and its fibre system was made from the seedling stage until maturity.

METHODS

The plants (*Linum usitatissimum* L. var. Liral Crown) were grown in 128 pots arranged in two groups, one in normal light in a glass-house (long day, summer conditions) and the other adjacent in the same glass-house and shaded with a hessian screen placed 4 ft. above the pots. Observations made with a photo-electric cell at the beginning of the experiment showed that the screen shaded all pots equally. Each group received two water treatments, 'normal' and 'drought'. There were four replicates of each of the four treatments, each replicate consisting of eight pots. The treatments are represented by the following symbols:

1. S_1W_1 —shaded, normal watering.
2. S_1W_2 —shaded, drought.
3. S_2W_1 —unshaded, normal watering.
4. S_2W_2 —unshaded, drought.

All plants were grown in enamel pots (11 in. diameter by 14 in. deep) containing 30 lb. of washed river sand. To each the following nutrients were added: 0.5 gm. N as $(\text{NH}_4)_2\text{SO}_4$; 2.0 gm. P_2O_5 as Na_2HPO_4 ; 1.0 gm. K as K_2SO_4 ; 0.37 gm. CaCl_2 ; 1.25 gm. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 4 mg. $\text{Fe}_2(\text{C}_4\text{H}_4\text{O}_6)_3$; 3 mg. MnCl_2 , and 0.4 mg. each of H_3BO_3 , CuSO_4 , and ZnSO_4 . Ten seeds were planted in each pot, the number of seedlings being reduced to four two days after emergence.

Eight samplings were made at fortnightly intervals from the time of emergence, each sample consisting of the plants from one pot taken at random from each replicate. One plant from each sampled pot was taken for anatomical study; the other three plants were used for growth analysis. The results are expressed as the mean of the plants in a sampled pot.

Continuous records of temperature, humidity, and evaporation were kept (Table I), and measurements of solar radiation were taken at intervals. Temperature and humidity were measured by thermographs and hygrographs respectively, solar radiation by an Ångström pyranometer, and evaporation as the amount of water lost from a filter-paper on a Buchner funnel attached to a burette. Unshaded plants received light ranging in intensity from 0.073 to 0.122 cal./sq. cm./min. on cloudy days, and from 0.219 to 0.584 cal./sq. cm./min. on sunny days; shaded plants received light of about 0.015 cal./sq. cm./min. on cloudy days, and from 0.029 to 0.088 on sunny days. As the experiment was carried out in the summer, the temperatures were higher and the light more intense and of a longer period than is usually found under normal growth conditions for flax. The 'normally watered' plants received sufficient water to keep the sand continually damp. The 'droughted' plants after the first sampling were given periodic droughts by allowing the sand to dry out to the wilting-point and then sufficient water added to bring the sand to its field capacity (see Table I).

TABLE I
Magnitude of Environmental Factors during Experimental Period

Period (weeks).	Mean day temp. (° F.).		Mean night temp. (° F.).		Mean day humidity (%).		Mean night humidity (%).		Mean evapora- tion (ml.).		No. of waterings.					
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_2	S_1	S_1W_1	S_1W_2	S_1W_3	S_2W_1	S_2W_2	S_2W_3
Sowing to emergence	79.6	79.6	68.1	68.1	60.6	60.6	77.5	77.5	76	54	1	1	1	2	2	2
Emergence-2	76.0	77.8	68.6	68.6	79.2	73.4	89.9	89.6	118	92	2	2	2	3	3	3
2-4 . . .	81.2	83.0	70.8	71.0	69.0	64.7	89.9	87.3	185	115	2	0	0	4	0	1
4-6 . . .	79.9	81.3	71.3	71.7	70.2	66.5	86.9	84.7	147	118	2	0	0	4	1	1
6-8 . . .	77.8	79.7	69.6	70.5	70.8	71.9	86.1	84.4	130	110	2	0	0	5	0	0
8-10 . . .	80.2	83.0	70.5	70.5	72.6	68.9	82.7	82.7	148	117	2	1	1	5	1	1
10-12 . . .	77.2	78.0	69.5	68.9	72.6	69.6	81.6	81.1	123	94	2	0	0	4	0	0
12-14 . . .	81.7	83.3	72.2	72.5	71.5	66.0	85.7	85.4	184	141	2	0	0	4	1	1
14-16 . . .	77.8	79.5	68.4	69.4	71.1	66.6	84.1	83.7	127	108	2	0	0	4	4	0

At each sampling, from the three plants used for growth analysis, determinations were made of fresh and dry weights, leaf area, water content, number of tillers, stem heights, and number of flowers and bolls. Leaf area was measured by a photo-electric cell method (Milthorpe, 1942). For a study of the fibre content material was taken from the base, middle, and apex only of the stem from the first three samplings of the unshaded pots, and the first five of the shaded pots; thereafter it was taken from immediately below the cotyledons, and at the base, one-quarter, one-half, three-quarters, and apex of the technical length.¹ The material was fixed in 70 per cent. formalin acetic alcohol and hardened in 70 per cent. alcohol. Sections were cut on a hand microtome, the material being held in pith. Two sections from each portion were stained with acid phloroglucin and mounted in glycerine. Total areas of stem, xylem, pith, phloem, pericycle, and outer cortex were obtained by tracing the images of the sections projected by an epidiascope on to paper, and measuring with a planimeter. Size of fibre cells was obtained by drawing, at a magnification of 950, their external boundaries, using five microscope fields from each section, with the aid of a camera lucida and measuring with a planimeter. Numbers of fibre cells, bundles, and cells per bundle were counted directly under a microscope. Fibre area was calculated from the product of the mean cross-sectional area and the number of fibre cells.

The number of lignified cells was taken as the number of fibre cells which stained with acid phloroglucin. Degree of lignification was obtained by ascribing to each stained cell a value of 1, 2, or 3 according to whether the middle lamella was $0-\frac{1}{3}$, $\frac{1}{3}-\frac{2}{3}$, or $\frac{2}{3}$ to wholly stained. The sum of these values multiplied by 100 and divided by 3 times the total number of fibre cells gave the degree of lignification expressed as a percentage.

RESULTS

Data from unshaded and shaded plants were analysed separately because of the nature of the experimental design and the wide differences in development. In all analyses of variance the sum of squares for the 'time' factor and its interactions was separated into three parts corresponding to the linear, quadratic, and cubic regression coefficients. Owing to lack of space only the analysis for dry weight is included. Differences found to be significant at the 5 per cent. level are specified as 'significant' in the text.

1. *Growth data*

Owing to the hot long-day conditions the unshaded plants grew rapidly and commenced flowering about 7 weeks after emergence. The shaded plants grew slowly and at the end of the experiment showed no traces of flowers.

(a) *Dry weight.* The dry weight of the shaded plants was much less than that of the unshaded plants. Under full light the droughted plants had a

¹ The technical length is here defined as that portion of the stem between the cotyledons and the subtending leaf of the inflorescence or between the cotyledons and the growing-point if the plant is not in flower.

lower dry weight than the normally watered plants; under shade there was no significant difference between the two water treatments (Table II). From an analysis of the logarithms of the dry weights of the unshaded plants (Table III)

TABLE II

Mean Dry Weight (gm.) of Plants from Different Treatments at Different Sampling Times

Weeks.	S_1W_1	S_1W_2	S_2W_1	S_2W_2
2	0.002	0.002	0.011	0.013
4	0.015	0.013	0.108	0.095
6	0.021	0.022	0.325	0.286
8	0.062	0.063	1.669	0.776
10	0.101	0.066	1.825	1.276
12	0.146	0.116	2.184	1.470
14	0.178	0.118	3.339	1.223
16	0.240	0.127	3.458	1.454

TABLE III

Analysis of Variance of Logarithms of Dry Weights of Unshaded Plants

Source of variance.	DF.	Sum of squares.	Mean square.
Blocks	3	0.355	0.118
Water	1	3.169	3.169*
Time	Linear	157.714	157.714†
	Quadratic	35.437	35.437†
	Cubic	1.974	1.974*
	Remainder	4	0.763
Blocks × Water	3	0.399	0.133
Blocks × Time	Linear	3	0.177
	Quadratic	3	0.200
	Cubic	3	0.195
	Remainder	12	1.246
Water × Time	Linear	1	1.852
	Quadratic	1	0.023
	Cubic	1	0.012
	Remainder	4	0.472
Blocks × Water × Time	Linear	3	0.021
	Quadratic	3	0.702
	Cubic	3	0.230
	Remainder	12	2.007
Total	63	206.948	

* Significance of variance ratio of term to its appropriate error mean square at 5 per cent. level; †, the same at 1 per cent. level.

it was found that the linear regression coefficient (measuring the relative growth rate), the quadratic regression coefficient (measuring the slackening of that rate), and the cubic regression coefficient for time were significant. Drought decreased the growth rate with time, although it did not accelerate the rate of that decrease; that is, the rate of falling-off in dry matter added per fortnight was not significantly less under drought than with normal water.

(b) *Leaf area.* Unshaded plants developed a greater leaf area than shaded plants (Fig. 1). The leaf area in both the shaded and unshaded treatments was significantly reduced by drought. The differences in leaf area between the shaded and unshaded plants were due both to a smaller number of leaves and to smaller leaves, while between water treatments the difference was due to number of leaves only (Table IV). This was largely a result of the more rapid

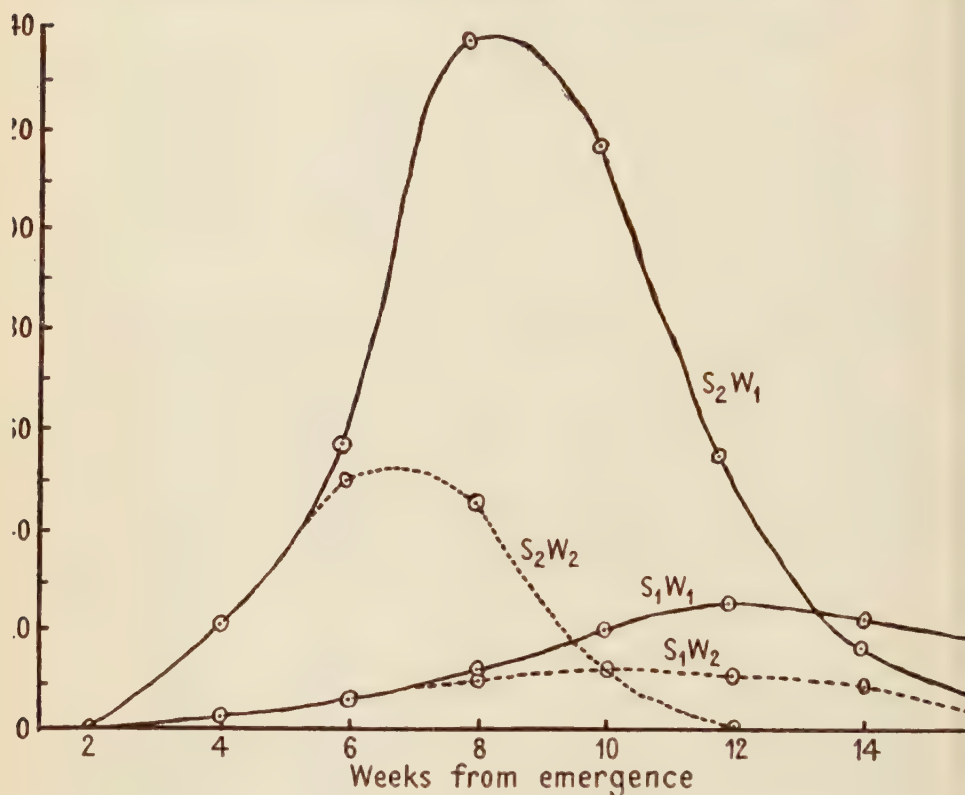


FIG. 1. Leaf area (sq. cm.) of plants from all treatments at all samplings.

dying-off of the lower leaves of the droughted plants, and partly because there were fewer tillers. There was no reduction in leaf size by drought. The maximum leaf area was attained earlier in the droughted treatments than with normal water, both in full light and under shade (Fig. 1). This was also a result of the earlier and greater dying-off of leaves in the droughted treatments.

(c) *Net assimilation rate.* The net assimilation rate was much lower under shade than in full light, but was not affected by drought (Table V). This is in keeping with the evidence put forward by Heath and Gregory that dry weight accumulation is not greatly influenced by climate. The figures for the mean net assimilation rate during the pre-flowering period are of the same order as those published by these workers for a number of plants from different areas.

TABLE IV
Area, Numbers, and Size of Leaves for all Treatments at all Samplings

Weeks.	Total leaf area (sq. cm.).						Total number of leaves.						Number of dead leaves.						Size of leaf (sq. cm.).					
	S_1W_1	S_1W_2	S_2W_1	S_2W_2	S_3W_1	S_3W_2	S_1W_1	S_1W_2	S_2W_1	S_2W_2	S_3W_1	S_3W_2	S_1W_1	S_1W_2	S_2W_1	S_2W_2	S_3W_1	S_3W_2	S_1W_1	S_1W_2	S_2W_1	S_2W_2	S_3W_1	S_3W_2
2	0.5	0.5	1.9	2.1	5	20	5	5	28	21	87	25	—	—	—	—	—	—	0.10	0.10	0.10	0.09	0.10	0.09
4	3.5	2.8	22.1	21.3	28	87	28	21	87	70	186	70	—	—	—	—	—	—	0.13	0.13	0.25	0.30	0.13	0.30
6	6.7	7.1	58.3	49.5	44	186	44	61	177	177	450	315	—	—	—	—	—	—	0.15	0.14	0.31	0.28	0.15	0.28
8	12.0	10.0	136.0	46.2	71	99	71	99	450	315	435	315	—	—	—	—	—	—	0.17	0.14	0.30	0.29	0.17	0.29
10	20.0	12.0	116.0	12.4	137	112	137	112	496	435	501	460	—	—	—	—	—	—	0.15	0.16	0.29	0.27	0.15	0.27
12	26.0	10.0	49.0	2.0	194	165	194	165	520	472	520	472	5	97	327	452	389	452	0.13	0.15	0.29	0.25	0.13	0.25
14	22.0	9.0	17.2	—	209	198	209	198	520	472	520	472	43	138	450	472	472	450	0.14	0.16	0.29	—	0.14	—
16	17.0	3.0	5.2	—	233	203	233	203	529	468	529	468	121	181	509	468	468	509	0.14	0.14	0.29	—	0.14	—

TABLE V

Net Assimilation Rates for all Treatments and Sampling Times
(gm. dry matter per sq. dm. per week)

Weeks.	S_1W_1	S_1W_2	S_2W_1	S_2W_2
0-2	—	—	—	—
2-4	0·192	0·190	0·591	0·495
4-6	0·173	0·173	0·392	0·386
6-8	0·154	0·151	0·365	0·344
8-10	0·153	0·153	0·320	0·385
10-12	0·112	0·103	0·431	0·441
12-14	0·088	0·043	0·505	0·440
14-16	0·060	0·037	0·260	—
Mean 2-10	0·168	0·167	0·417	0·403

(d) *Number of tillers.* The number of tillers which developed was reduced both by shading and by drought. The mean numbers per plant at 16 weeks were: S_1W_1 , 1·0; S_1W_2 , 0·9; S_2W_1 , 2·0; S_2W_2 , 1·6. The difference between the two water treatments under shade was not significant. The significant difference between the two water treatments under full light was due to the larger number dying in the droughted treatment. The number of tillers initiated was not affected by drought. There was on the other hand a greater initiation of tillers in the unshaded than in the shaded plants.

(e) *Height.* For observations on growth in height various portions of the stem were measured separately. They are defined as follows: (a) Total height is the length from ground level to the top of the main stem, or flowering stem if present; (b) technical length is that portion between the cotyledons and the subtending leaf of the inflorescence (or growing-point if not flowering); (c) length of flowering stem is the length of the panicle, from the leaf subtending the inflorescence to the top of the uppermost flower or boll; (d) total tiller length is the product of the mean length of a single tiller and the number of tillers.

Unshaded plants attained a greater total height than shaded plants (Table VI). Under shade no significant difference between the total height of plants from the two water treatments was recorded. Under full light drought gave plants of a lower total height than normal water: this was a result of a shorter flowering stem. There was no difference in the technical lengths from the two treatments. Total tiller length was also reduced by drought, there being a significant decrease in numbers of tillers, tiller technical length, and tiller flowering stem. Under shade there was no significant difference in total tiller length between the water treatments. The significant effect of drought on the flowering stem and tiller length of the unshaded plants exemplifies the progressive effect of reduced water supply on growth with time.

(f) *Flowers and bolls.* A significantly lower number of bolls was produced in the droughted than in the normally watered plants (Table VII). This could be accounted for by the fact that fewer flowers were initiated and of these a far lower proportion developed into bolls. Although seed weights or counts

TABLE VI
Total Height, Technical Lengths and Lengths of flowering Stems for all Treatments and Samplings (cm.)

Weeks.	Total height of main stem.						Total tiller length.						Technical length of main stem.						Flowering stem.			Tiller.	
	S_1W_1	S_1W_2	S_1W_3	S_2W_1	S_2W_2	S_2W_3	S_1W_1	S_1W_2	S_1W_3	S_2W_1	S_2W_2	S_2W_3	S_1W_1	S_1W_2	S_1W_3	S_2W_1	S_2W_2	S_2W_3	S_1W_1	S_1W_2	S_1W_3	S_2W_1	S_2W_2
2	5'3"	5'4"	10'0"	12'2"	12'2"	12'2"	—	—	—	1'3"	1'1"	7'2"	9'1"	—	—	—	—	—	—	—	—	—	—
4	13'0"	14'4"	23'5"	26'3"	26'3"	26'3"	—	—	—	9'4"	1'9"	21'1"	23'5"	—	—	—	—	—	—	—	—	—	—
6	25'4"	23'7"	54'9"	52'9"	52'9"	52'9"	—	—	—	21'7"	19'7"	52'2"	50'5"	—	—	—	—	—	—	—	—	—	—
8	45'3"	39'7"	99'5"	78'5"	78'5"	78'5"	3'0"	3'8"	3'8"	40'7"	36'0"	89'0"	74'7"	—	—	—	—	—	—	—	—	—	—
10	52'0"	49'8"	111'4"	93'7"	93'7"	93'7"	15'4"	14'5"	14'5"	49'2"	45'4"	91'7"	79'5"	8'1"	1'8"	—	—	—	—	—	—	—	—
12	68'6"	58'6"	122'9"	100'7"	100'7"	100'7"	33'5"	24'7"	209'7"	64'6"	55'3"	96'6"	83'0"	17'1"	11'6"	11'1"	15'0"	15'0"	17'5"	15'1"	15'1"	11'1"	5'0"
14	71'3"	63'5"	128'6"	109'7"	109'7"	109'7"	42'8"	32'3"	246'8"	66'9"	60'2"	96'2"	90'6"	30'4"	14'5"	30'4"	14'5"	14'5"	30'4"	14'5"	14'5"	19'7"	5'4"
16	85'7"	63'8"	132'1"	109'5"	109'5"	109'5"	58'1"	38'1"	247'5"	81'5"	59'8"	95'5"	90'7"	31'4"	16'5"	31'4"	16'5"	16'5"	31'4"	16'5"	16'5"	27'7"	8'9"

were not recorded, it was noted that the seed from the droughted plants was smaller and very pinched. These observations, together with the boll counts, demonstrate the deleterious effect of drought on seed production.

TABLE VII

Number of Flowers opened and Bolls produced in Plants under Normal Light

Weeks.	Flowers opened.		Number of bolls.		Ratio bolls to total flowers.	
	S_2W_1	S_2W_2	S_2W_1	S_2W_2	S_2W_1	S_2W_2
8	2	1	—	—	0·00	0·00
10	19	10	4	3	0·21	0·30
12	31	18	12	4	0·39	0·22
14	47	21	20	3	0·43	0·13
16	48	21	25	5	0·52	0·24

2. Quantitative development of fibre

(a) *Fibre content.* The volume of stem and volume of fibre were calculated as the product of the respective mean cross-sectional area and the technical length. The percentage fibre by volume was obtained from these figures. The unshaded plants had a higher percentage of fibre than the shaded plants (Table VIII). Drought had no significant effect on this percentage either in

TABLE VIII

Percentage of Fibre (by volume) in all Treatments at all Samplings

Weeks.	S_1W_1	S_1W_2	S_2W_1	S_2W_2
2	0·41	0·32	2·40	2·14
4	1·30	1·08	2·55	3·61
6	2·00	1·87	4·39	4·90
8	2·09	1·58	5·10	5·92
10	2·91	3·73	6·08	6·12
12	3·50	3·21	5·97	5·38
14	3·14	3·42	5·18	4·80
16	3·13	3·14	4·80	4·21

full light or in shade. The percentage of fibre increased until the tenth week (i.e. after the commencement of flowering) in the unshaded plants and until the twelfth week in the shaded plants. It then decreased markedly in the unshaded plants.

This was obviously due to differences in the development of the stem and fibre tissues. In the unshaded plants the volume of fibre developed almost exponentially up to the time of flowering. The rate of development then decreased especially under drought (Fig. 2). Under drought the volume of fibre did not increase after the tenth week, but it did increase in the normally watered plants up to full maturity. The amount of fibre which developed in these plants from flowering until full maturity comprised 19 per cent. of the total fibre produced. In the normally watered plants the total volume of stem tissue increased almost linearly from emergence until full maturity

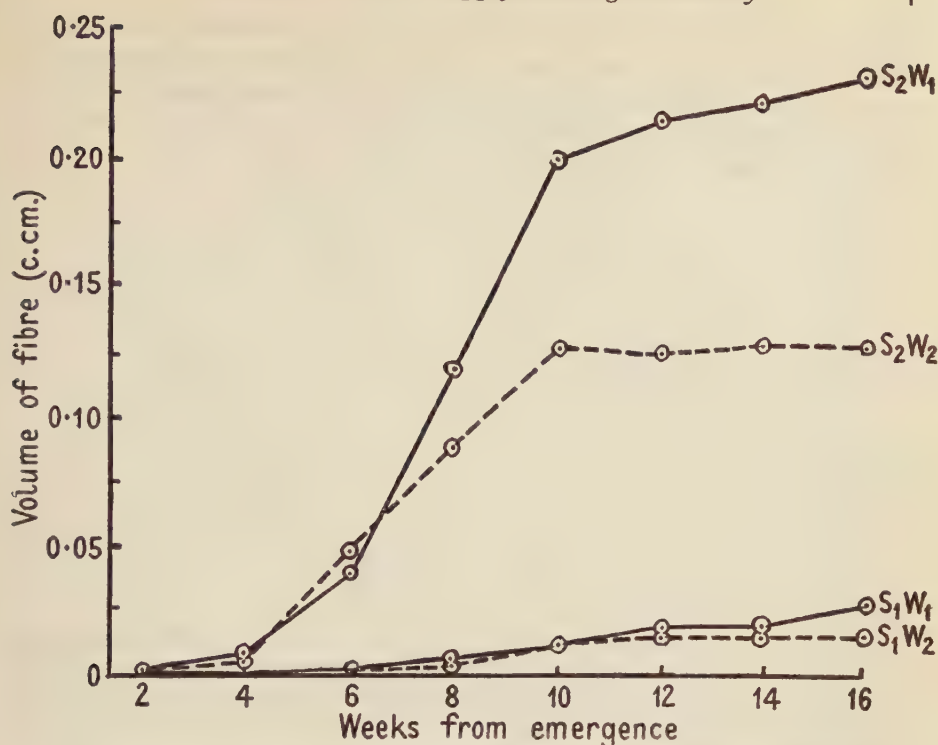


FIG. 2. Volume (c.c.) of fibre in stem of plants from all treatments at all samplings.

TABLE IX

Mean Cross-sectional Area of Fibre and of Total Stem Tissues of Unshaded Plants for all Samplings. (sq.mm. $\times 10^{-2}$)

Weeks.	Fibre.		Total stem tissue.	
	S_2W_1	S_2W_2	S_2W_1	S_2W_2
2	1.50	1.48	40.3	38.9
4	3.51	2.75	119.9	119.5
6	7.55	9.45	189.3	174.1
8	13.40	11.95	256.9	202.5
10	21.79	15.81	332.4	282.7
12	22.21	13.93	374.8	289.4
14	23.12	15.72	474.4	297.8
16	24.28	13.70	552.2	307.5

(Fig. 3) In the droughted plants the volume of stem tissue increased until full maturity, but the rate of increase was less, especially after flowering. The volume of stem tissue developed after the tenth week represented 21 per cent. in the droughted and 40 per cent. in normally watered plants of the total amounts produced.

The smaller volume of fibre in the droughted than in the normally watered plants was due to the smaller cross-sectional area of fibre (Table IX). There

was no difference in stem length. Similarly, the smaller volume of total stem tissue of the droughted plants was due to the smaller cross-sectional area of stem. Drought reduced growth in diameter to a greater extent than in length; that is, it had an earlier and greater effect on the activity of the cambium than on the apical meristem.

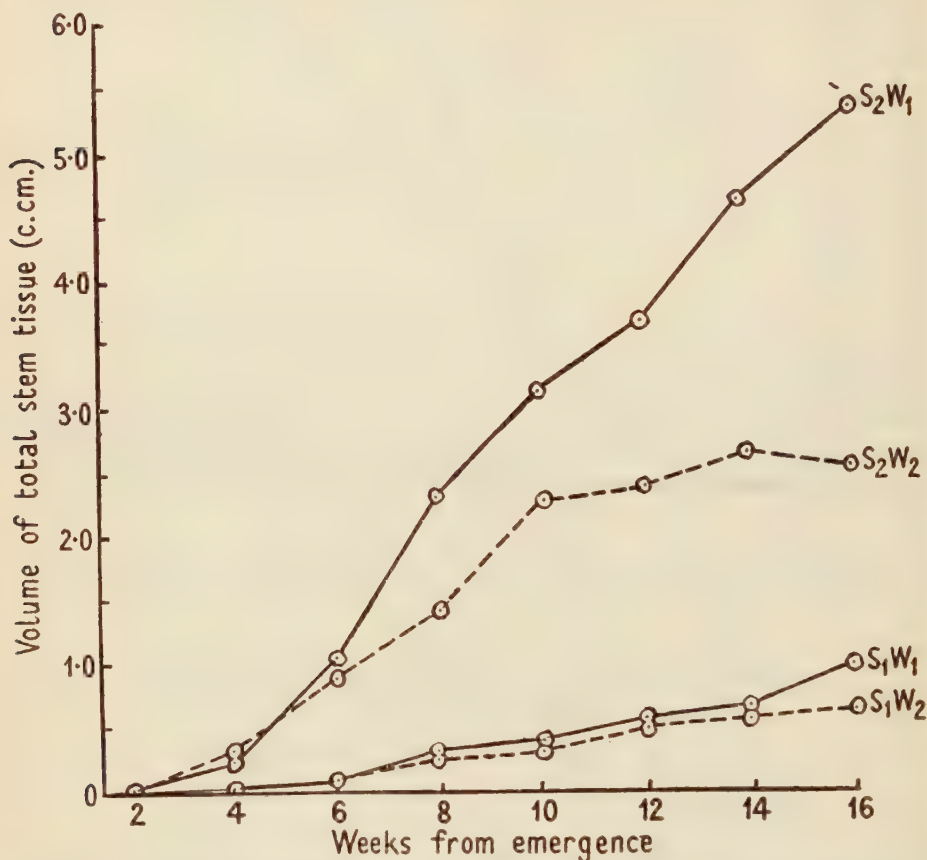


FIG. 3. Volume (c.c.) of total stem tissue per plant from all treatments at all samplings.

Under shade the differences in volume of fibre and of stem between the two water treatments were not significant.

(b) *Number of fibre cells.* Fewer fibre cells developed in the shaded than in the unshaded plants (Table X). This was consistent with the smaller stems of the former. In the unshaded plants there was no significant difference between the two water treatments in the number of fibre cells in the lower half of the technical length. In the upper portions of the technical length there were significantly fewer fibre cells in the droughted than in the normally watered plants (Fig. 4). The number of fibre cells which developed varied both with time and with the stem level. There were fewer fibre cells below and just above the cotyledons than at all other stem levels. There was an

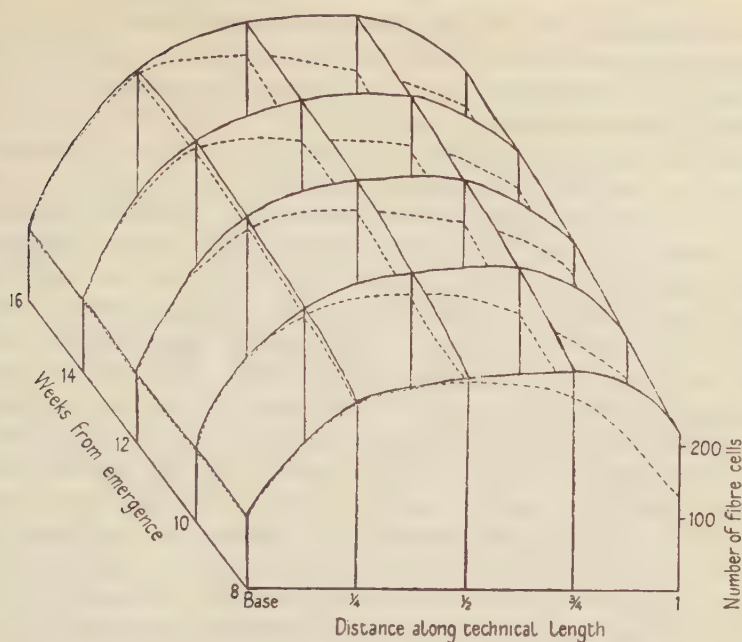


FIG. 4. Diagram of a three-dimensional model showing number of fibre cells per cross-section at different stem levels of unshaded plants for samplings from 8 to 16 weeks. (Continuous lines indicate normally watered plants and broken lines droughted plants.)

TABLE X

Number of Fibre Cells at Base and Middle of Technical Length at Different Times

Weeks.	Base.				Middle.			
	S_1W_1	S_1W_2	S_2W_1	S_2W_2	S_1W_1	S_1W_2	S_2W_1	S_2W_2
2	30	28	42	44	40	41	58	61
4	24	27	64	67	54	52	123	121
6	32	22	99	92	76	78	228	215
8	26	25	93	108	93	68	279	294
10	42	51	112	89	77	120	347	306
12	39	30	90	97	112	104	346	363
14	28	42	113	100	110	129	364	342
16	47	49	113	96	131	125	342	343

increase in the number of fibre cells with stem level, from the base to three-quarters of the technical length. At the apex there were fewer fibre cells than at the lower levels, with the exception of the base. The number of fibre cells at the base of the technical length increased with time until flowering; thereafter there was no further increase (Table X). At the middle of the stem the number increased linearly and very steeply up to the time of flowering, after which time the increase was slow. At the other stem levels the number of fibre cells continued to increase after flowering, the increase being greater in the upper than in the lower portions of the stem (Fig. 4).

Under shade there was no significant difference between the two water treatments in the number of fibre cells at any one stem level. The increase in number with time and with stem level followed a similar trend to that of the normally watered unshaded plants.

The increase in number of fibre cells per cross-section at the same stem level was due to the fact that certain potential fibre cells do not show secondary deposition of cellulose until late in development. Sections from material taken at times right up to full maturity showed numerous large, irregular, and hyaline cells on the inner side of depositing cells. These cells were similar to those found in the bast region of the apical meristem before deposition had commenced and were larger than the normal parenchyma cells which separate the fibre bundles; they were probably fibre cells in which deposition of cellulose had not commenced. Tiver (1942) has supported this view. Krabbe (1887) and Searle (1923), however, have attributed an increase in the number of fibre cells after flowering to the sliding growth of these cells past one another as they elongate; Anderson (1927) apparently subscribed to the same view. Tammes (1907) denied the occurrence of sliding growth in the bast fibres of flax. While sliding growth may contribute to the increase in the number of fibre cells per cross-section, the delayed development of certain fibre cells is in the opinion of the writer the chief factor causing this increase.

(c) *Cross-sectional area of fibre cells.* The cross-sectional area of a single fibre cell was smaller in the shaded than in the unshaded plants (Table XI).

TABLE XI

Cross-sectional Area of Fibre Cells at Base and Middle of Technical Length in all Treatments at all Samplings. (sq.mm. $\times 10^{-4}$)

Weeks.	Base.				Middle.			
	S_1W_1	S_1W_2	S_2W_1	S_2W_2	S_1W_1	S_1W_2	S_2W_1	S_2W_2
2	2.56	2.48	3.04	3.08	0.90	0.92	2.52	2.41
4	5.08	5.21	6.62	7.80	1.26	1.42	4.07	3.33
6	6.15	7.69	15.17	18.51	1.80	2.30	4.02	4.35
8	8.85	8.09	30.93	23.95	2.99	1.97	4.37	4.01
10	8.14	9.86	25.91	19.36	4.12	3.05	6.40	5.28
12	6.93	8.64	23.31	18.46	3.74	3.32	6.53	4.58
14	9.08	6.75	22.41	16.03	3.77	3.48	6.56	5.33
16	7.01	7.27	19.92	15.43	3.51	2.70	7.35	4.43

In the unshaded plants drought significantly decreased the cell size in all positions of the technical length (Fig. 5). The cell size decreased from the base to the apex of the technical length. They were very much larger below and just above the cotyledons than elsewhere in the stem. With time the basal cells increased in size up to flowering; after flowering they became smaller owing to shrinking, which was as marked with a plentiful supply of water as with drought. The fibre cells at the middle of the technical length continued to increase in area until maturity in plants with normal watering, but there was no increase after flowering in the droughted plants.

No measurement was made of the amount of cellulose deposited in the

fibre cells, though observations indicated that it was greater in the normally watered than in the droughted plants. The increase in cross-sectional area after flowering may be due either to a growth in circumference of the cells or to the fact that other cells commencing to show secondary deposition of cellulose are larger than those in which deposition has already begun.

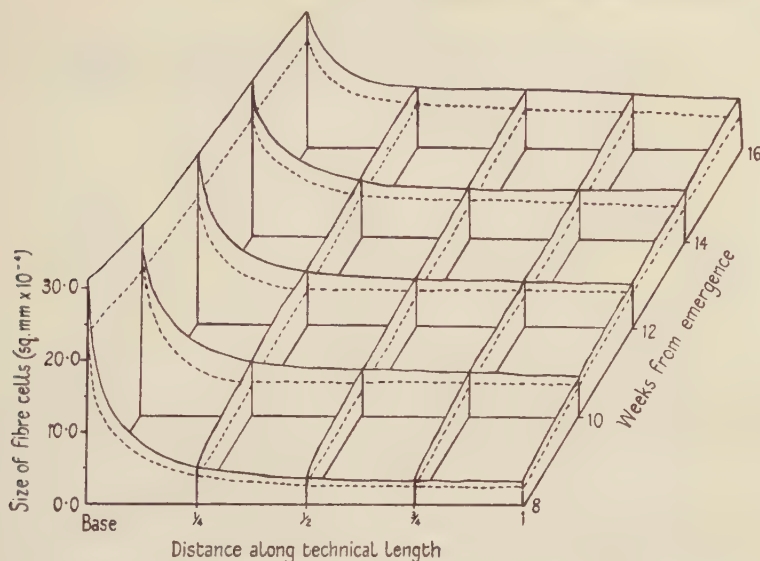


FIG. 5. Diagram of a three-dimensional model showing cross-sectional area (sq.mm. $\times 10^{-4}$) of fibre cells at different stem levels of unshaded plants for samplings from 8 to 16 weeks. (Continuous lines indicate normally watered plants and broken lines droughted plants.)

Under shade there was no difference in the cross-section area of fibre cells between the two water treatments. The size of fibre cells increased with time and decreased with distance above the cotyledons in a similar manner to that of the unshaded, normally watered plants.

(d) *Fibre bundles.* There was no difference in the number of fibre bundles per cross-section between the shaded and unshaded plants nor between the two water treatments in either group (Table XII). There were fewer fibre

TABLE XII

Number of Fibre Bundles at Base and Middle of Technical Length in all Treatments

Weeks.	Base.				Middle.			
	S_1W_1	S_1W_2	S_2W_1	S_2W_2	S_1W_1	S_1W_2	S_2W_1	S_2W_2
2	25	24	24	22	39	41	44	41
4	24	27	18	20	42	41	44	41
6	24	14	25	19	42	39	45	46
8	16	18	24	22	40	40	47	42
10	24	22	27	24	39	39	42	47
12	23	17	21	24	41	39	44	43
14	16	24	24	22	40	42	42	37
16	23	25	24	24	40	40	44	41

bundles per cross-section of stem below and just above the cotyledons than at the higher stem levels, but there was no difference in the number of fibre bundles with stem level from a distance of one-quarter of the technical length to the apex (Table XIII). The number of fibre bundles at any one position

TABLE XIII

Number of Fibre Bundles in Different Treatments at Different Levels of Technical Length. (The data of shaded plants relate to 14-16 week samplings, those for unshaded plants to 8-16 week samplings)

Distance along technical length.	S_1W_1	S_1W_2	S_2W_1	S_2W_2
Below cotyledons	19	18	19	20
Base . . .	23	22	23	20
$\frac{1}{4}$. . .	38	41	41	42
$\frac{1}{2}$. . .	40	40	43	44
$\frac{3}{4}$. . .	40	41	43	42
1 . . .	—	—	46	44

of the stem remains constant throughout the growth period. During this time there was a large increase in the number of fibre cells. Cells commencing to show cellulose deposition adjoin fibre cells in which there was previous deposition of cellulose. That is, secondary deposition of cellulose commenced in some cells of all fibre bundles at the time of emergence. The increase in the number of fibre cells with time resulted not in an increase of fibre bundles but in an increase in the number of cells per bundle (Fig. 6).

(e) *Lignification of fibre cells.* The fibre cells of the unshaded plants were much more heavily lignified than those of the shaded plants (Table XIV).

TABLE XIV

Degree of Lignification of Fibre Cells at Base and Middle of Technical Length of all Treatments at all Samplings

Weeks.	Base.				Middle.			
	S_1W_1	S_1W_2	S_2W_1	S_2W_2	S_1W_1	S_1W_2	S_2W_1	S_2W_2
2	—	—	—	—	—	—	—	—
4	—	—	3.4	3.5	—	—	0.5	0.5
6	—	—	5.0	4.0	—	—	1.6	1.9
8	—	—	5.8	6.2	—	—	2.2	2.0
10	0.6	1.6	6.1	4.4	—	—	3.2	1.0
12	—	2.2	10.7	8.9	0.3	1.9	7.4	4.0
14	2.0	4.6	7.7	6.9	0.6	1.0	5.7	4.2
16	3.5	2.7	12.0	9.2	0.3	0.5	6.6	2.3

In the unshaded plants, while the effect of drought as a whole was not significant, the interaction between water and stem level was highly significant, the fibre cells below the cotyledons being more heavily lignified in the droughted than in the normally watered plants (Table XV). There were no differences between treatments at the base of the technical length, but the fibre cells at the higher stem levels were more lignified in the normally watered than in

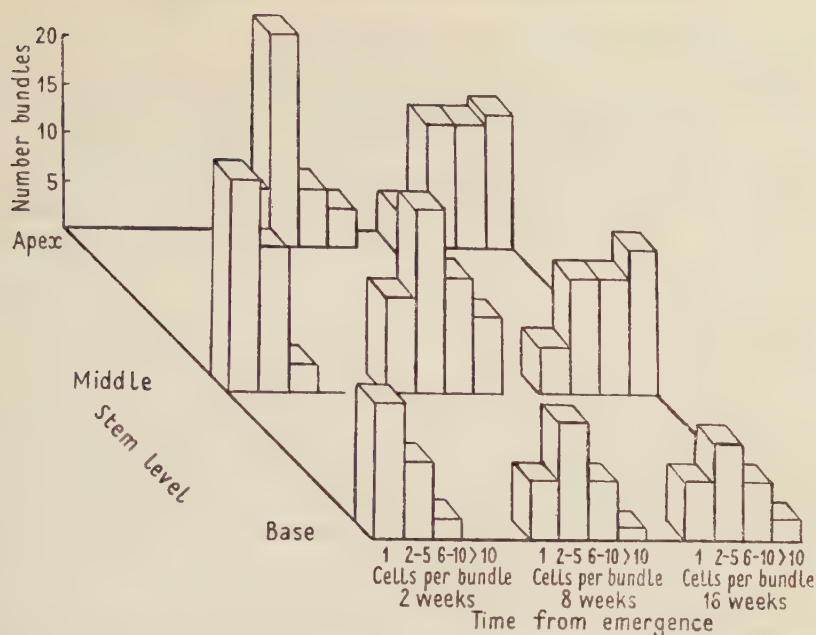


FIG. 6. Diagram of a three-dimensional model showing frequency of fibre bundles with different numbers of cells at the base, middle, and apex of the technical length of normally watered unshaded plants at samplings at 2, 8, and 16 weeks from emergence.

TABLE XV

Degree of Lignification at various Positions on Technical Length of all Treatments. (Means of readings from samplings 10-16 weeks)

Distance along technical length.	S_1W_1	S_1W_2	S_2W_1	S_2W_2
Below cotyledons	6.2	4.7	18.9	23.9
0 . . .	1.5	2.8	9.9	7.3
$\frac{1}{4}$. . .	0.6	0.4	2.6	1.7
$\frac{1}{2}$. . .	0.3	0.8	4.5	2.6
$\frac{3}{4}$. . .	0.4	0.4	7.3	3.1
1 . . .	—	—	5.2	3.0

the droughted plants. The cells below and just above the cotyledons were more heavily lignified than at other stem levels. With time there was an increase in the degree of lignification at all stem levels until flowering; thereafter there was no increase at any level. The greater lignification in the unshaded normally watered plants than in the droughted plants appeared to be due to a larger number of cells showing lignin deposition rather than to a greater amount of lignin being deposited in a similar number of cells, as there were a greater number of lignified cells in the normally watered than in the droughted plants. On the other hand, because the fibre cells were larger in the normally watered than in the droughted plants, a greater deposition of

lignin would be necessary in the former to give an equal degree of lignification, if an equal number of cells were lignified.

Under shade there was no difference between the two water treatments in the degree of lignification of fibre cells. The cells below and just above the cotyledons were again more heavily lignified than at other stem levels.

(f) *Variations in relative proportions of different parts of the stem during development.* The observations in this section are confined to results from the unshaded, normally watered plants, which showed no differences from the droughted plants in the relative proportions of the different stem tissues. The volumes of all tissues were reduced proportionately by drought. No data were collected on the number and size of cells other than fibre cells. The main object is to show how the relative amounts of the different tissues vary during growth at different positions of the stem.

There was a gradual increase with time in the percentage of xylem at all parts of the stem, the phloem remained fairly constant, while the percentage of pith, pericyclic tissue, and outer cortex decreased (Fig. 7). That is, the chief growth in diameter of the stem was due to increase in the xylem, whilst the phloem expanded sufficiently to maintain a constant proportion. The decrease in the other portions was due to the fact that the actual amounts remained constant while the xylem and phloem expanded. The fibre occupied a continually greater proportion of the pericycle as the plant grew, at all positions except the base.

Similar variations occurred at different stem levels (Fig. 8). There was a far greater proportion of xylem relative to other tissues at the base of the stem; this decreased towards the apex. The proportion of pith increased greatly, the phloem and pericycle decreased slightly, and the outer cortex increased from the base to the apex. The relative proportion of fibre was greatest at the base at flowering but least at maturity. This was due to the shrinkage of the undeveloped fibre cells (section 2 (c)). At other positions, while the percentage of fibre decreased from the base to the apex at flowering, there was no significant difference at maturity.

DISCUSSION

Compared with plants grown in full light the shaded plants had a lower dry weight and height, a higher water content, a lower net assimilation rate, fewer tillers, and a smaller volume of fibre and stem tissue. Although these effects can be attributed to lack of light, they are not solely the result of the lower assimilation rate. Some secondary deposition of cellulose took place in the fibres which would not be expected if the plants were suffering from carbohydrate starvation. It appears then that the lower degree of growth and development of the shaded plants is due to some effect of light other than that which it exerts on assimilation; a phenomenon also noted by Ashby and Oxley (1935) for *Lemna*. The effect of water was less striking than that of light. Under unshaded conditions drought resulted in many important

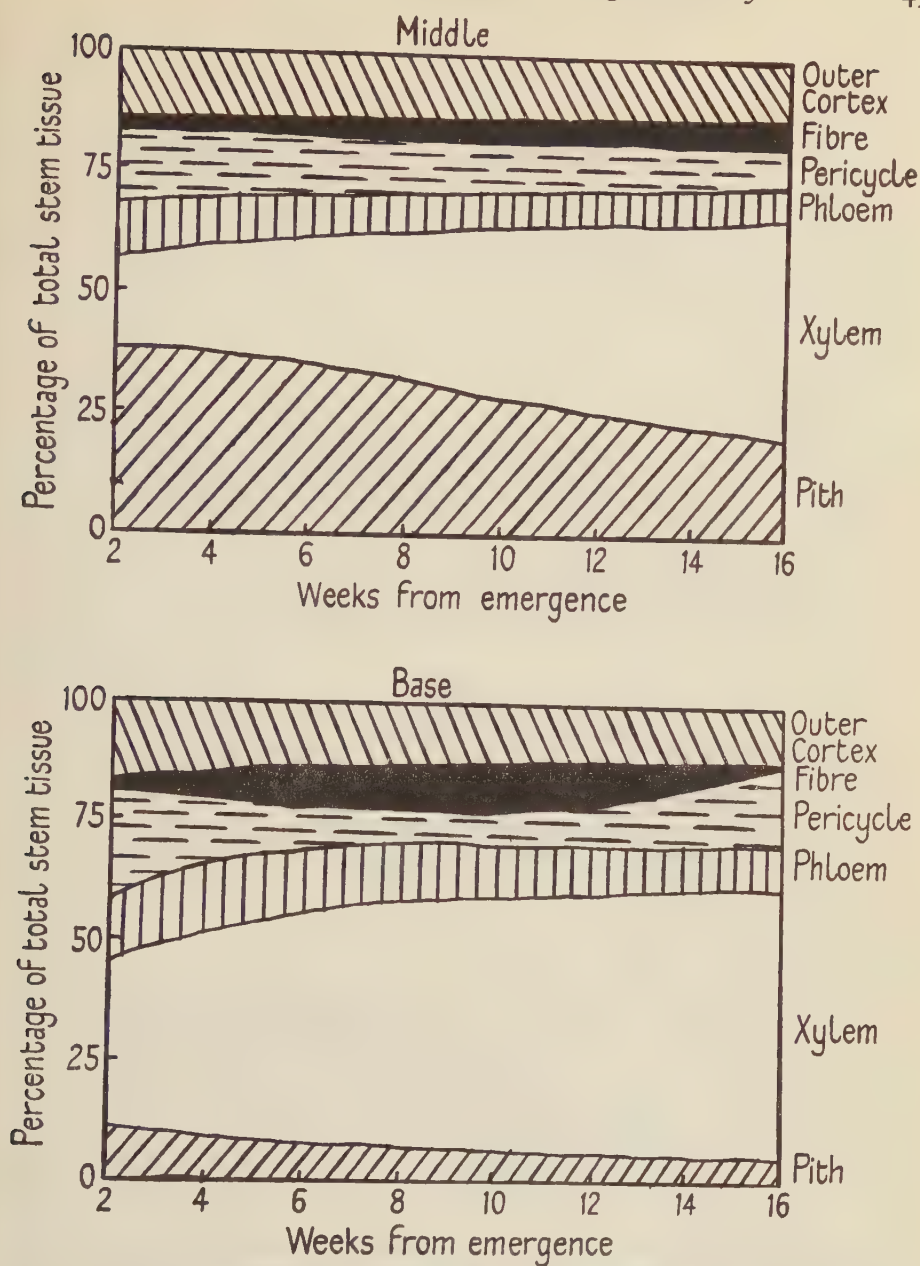


FIG. 7. Relative proportions of cross-sectional areas of different stem tissues at base and middle of the technical length of normally watered unshaded plants for all samplings.

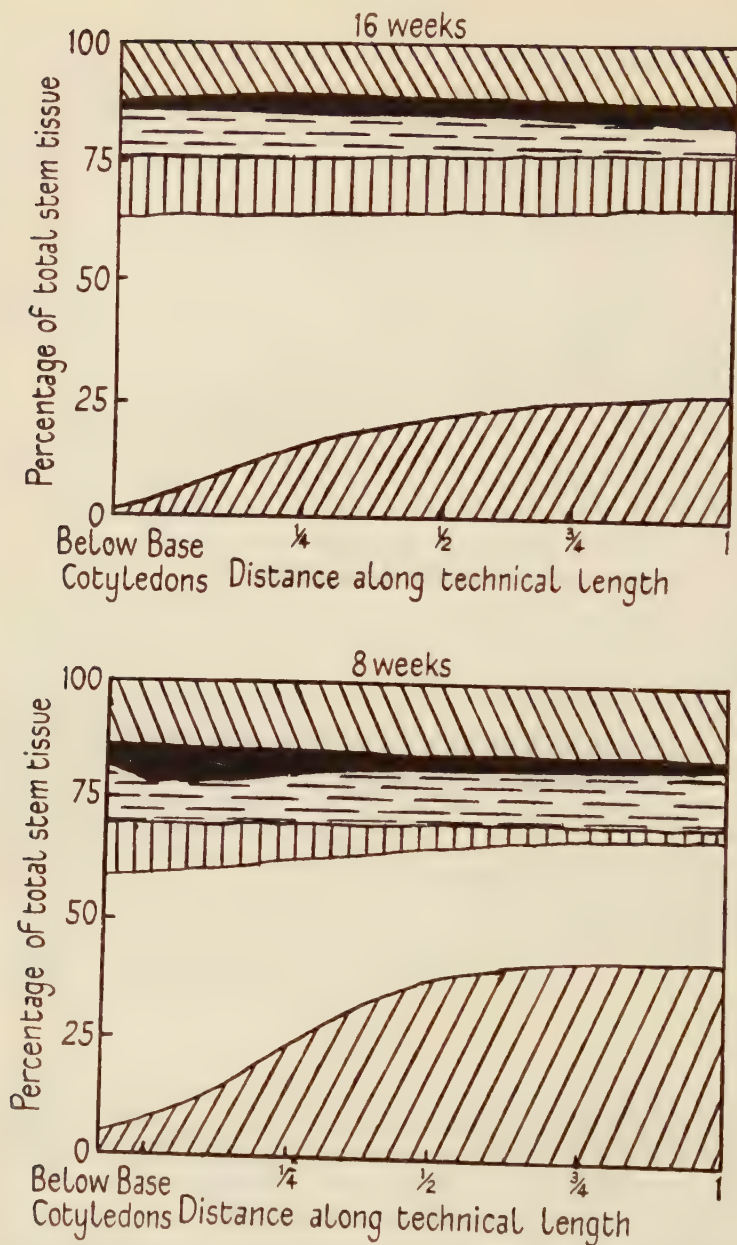


FIG. 8. Relative proportions of different stem tissues at different positions on the technical length of normally watered unshaded plants at samplings at 8 weeks and 16 weeks after emergence. (Tissue indications as in Fig. 7.)

differences, but with shading the effects of drought were not significant. This was probably due to lower transpiration, which meant that an actual effect of drought was not experienced.

In full light drought resulted in a marked decrease in the growth of the plant, as measured by dry weight, height, &c. Its effect was naturally more pronounced toward the end of the experiment, as can be seen in the striking reduction in length of flowering stem and numbers of flowers and bolls. In general periodic drought caused deleterious effects, resulting in smaller plants, and thus a lower straw weight, and fewer bolls and seeds. The lower dry weight was not due to a decreased rate of assimilation, but a smaller leaf area caused by the earlier death of a large number of leaves under drought conditions.

The proportion of fibre to total stem increased up to the time of flowering, and then fell off. This was due to the fibre increasing more rapidly than the other tissues before flowering, after which time there was a rapid falling-off in the amount of fibre added, whereas the other stem tissues continued to increase. This increase in stem tissue was due solely to cambial growth of the stem, which was much less under drought than with normal water. The actual volume of fibre was also greatly reduced by drought. Under shading there was less development of fibre and stem tissue than under normal light and the percentage fibre was also lower, indicating that the fibre was reduced to a greater extent than was the other tissue.

Shading reduced fibre volume by shortening the stem and reducing the mean transverse area of fibre in the stem. This latter reduction was attributable to both the smaller number and smaller size of fibre cells. Under unshaded conditions drought reduced the fibre volume by decreasing the size of the fibre cells. The larger fibre cells in the normally watered than in the droughted plants may be due to one or both of two reasons: (a) there may be an increase in the cross-sectional area of certain cells, as has been observed by Anderson (1927). This increase in area may be restricted by reduced water supply or by reduced light. (b) It is also probable that cells which begin to deposit cellulose late in the development of the plant may be larger than cells which commence secondary cellulose deposition earlier. These potential fibre cells may not reach the same dimensions in the droughted plants as similar cells in normally watered plants. We do know, however, that there is a progressive increase with time in the mean cross-sectional area of individual fibre cells under normal light and water supply, and that this increase is restricted by drought and by shading.

The number of fibre bundles at any one stem level remained approximately constant throughout the life of the plant. Cells in which cellulose deposition was beginning were added to pre-existing bundles. This resulted in an increase in the mean number of cells per bundle. The number of cells per bundle varied greatly with the position in the stem. The smaller number of bundles at the base of the stem, together with the large cell size, the smaller number of cells, and the greater lignification of these cells, indicates that this

fibre is of little importance commercially. It would all be eliminated during scutching as 'tow'. The best line fibre comes from the upper portions of the stem which have a large number of bundles of small, lightly lignified cells. The contention that pulling yields more line fibre than cutting is inconsistent with these results. It seems unlikely that the yield of line fibre at least would be greater from pulled than from cut flax, especially where close cutting is possible. The height above ground level at which the poor fibre gave place to better quality fibre was not determined, but at one-quarter of the technical length (about 8 inches) the fibre was quite good.

ACKNOWLEDGEMENTS

My sincere thanks are due to Professor E. Ashby, under whose direction the work was carried out, for his keen interest and assistance during the progress of the study and in the preparation of the manuscript. I am greatly indebted to Miss Helen Turner for statistical advice.

I gratefully acknowledge the generosity of the Walter and Eliza Hall Trust for providing the funds for this work.

SUMMARY

An experiment is described in which the quantitative anatomy of the flax plant was studied from emergence to maturity under four conditions of light intensity and water supply.

The plants grown under shade in a glass-house were much less developed than those grown in the same house without shade. They were shorter, and had a lower dry weight, smaller leaf area, higher water content, lower yield and percentage of fibre, lower number of fibre cells, and smaller fibre cells. These shaded plants did not flower.

Under shade there were no significant differences between plants grown under the two water treatments. It is likely that transpiration was too low for there to have been an effective drought.

Under full light in the glasshouse drought resulted in a lower dry weight, shorter plants (mainly by reducing the length of the flowering stem), a smaller leaf area, less development of flowers and bolls, a lower yield of fibre but not a lower percentage of fibre, and fibre cells of smaller size.

Fibre cells at the base of the plant were few in number but were very large. They increased in size throughout growth until flowering, after which they decreased as a result of shrinkage. The fibre cells higher in the stem were much smaller than at the base, but they were far more numerous and were closely packed. These are the requirements of good quality fibre. The cells at the higher stem levels increased in size and number right up to maturity.

The number of fibre bundles at any one position in the stem remained constant throughout the growth of the plant. The increase in number of cells showing secondary deposition resulted in the addition of cells to previously existing bundles. The number of bundles at the base was less than at higher levels of the stem.

Fibre cells at the base of the stem were more heavily lignified than cells at higher stem levels, as estimated by acid phloroglucin. / At all stem levels, except at the base, fibre cells in plants receiving normal water were more heavily lignified than those in droughted plants.

LITERATURE CITED

- ANDERSON, D. B., 1927: A Microchemical Study of the Structure and Development of Flax Fibres. *Amer. Journ. Bot.*, xiv, 187-211.
- ASHBY, E., and OXLEY, T. A., 1935: The Interaction of Factors in the Growth of Lemna. VI. An Analysis of the Influence of Light Intensity and Temperature on the Assimilation Rate and the Rate of Frond Multiplication. *Ann. Bot.*, xlix, 309-36.
- FISHER, R. A., 1941: Statistical Methods for Research Workers. London. 8th edition.
- HEATH, O. V. S., and GREGORY, F. G., 1938: The Constancy of the Assimilation Rate and its Ecological Importance. *Ann. Bot.*, N.S., ii, 811-18.
- KRABBE, G., 1887: Ein Beitrag zur Kenntniss der Structur und des Wachstums vegetabilischer Zellhäute. *Jahrb. d. wiss. Bot.*, xviii, 346-423.
- MILTHORPE, F. L., 1942: A Simplified Photo-electric Cell Method for measuring Leaf Areas. *Journ. Aust. Inst. Agric. Sci.*, viii, 27.
- SEARLE, G. O., 1923: A Botanical Study of the Flax Plant. III. Some Observations on the Development of the Stem and its Fibre. *Linen Ind. Res. Assoc. Memoir*, xiii.
- , 1928: A Botanical Study of the Flax Plant. IX. The Fibre System of the Stem. *Linen Ind. Res. Assoc. Memoir*, li.
- TAMMES, T., 1907: *Der Flachsstengel*. Haarlem. (Quoted by Tobler, 1928.)
- TIVER, N. S., 1942: Studies of the Flax Plant. I. Physiology of Growth, Stem Anatomy, and Fibre Development in Fibre Flax. *Aust. Journ. Exp. Biol.*, xx, 149-60.
- TOBLER, F., 1928: *Der Flachs als Faser- und Ölpflanze*. Berlin.

Oxidation Systems in the Potato Tuber

BY

J. G. BOSWELL

(*The University, Sheffield*)

With six Figures in the Text

INTRODUCTION

IT has been shown previously by Boswell and Whiting (1938) that more than one oxidase is concerned in the oxygen uptake by slices of potato tuber. Baker and Nelson (1943) using a similar technique reached the same conclusion, while Steward and Preston (1940) investigating a different problem were nevertheless brought to a similar view. In all cases the greater part of the oxygen uptake was dependent upon the catechol oxidase system, while one or more other systems were concerned in not more than a residual 30 per cent. of the uptake. The results of a further investigation of the problem are recorded in this paper. Part I is concerned with a detailed examination of the polyphenol oxidase system, in particular its ability to oxidize a number of phenolic compounds; part II with observations on the oxidation of dihydroxymaleic acid; part III with the oxidation of ascorbic acid, while part IV contains observations on the capacity of the tissue to oxidize organic acids and amino acids. The relationship between these acids and the polyphenol oxidase system is considered.

METHODS

The preparation of the tissue and the measurements of respiration rates were carried out using the methods described by Boswell and Whiting (1938). Except where otherwise stated the slices were 0.03 in. in thickness and were immersed in 5 c.c. phosphate buffer, pH 5.5, 0.026 M concentration. The temperature of the thermostat was 23° C. The variety of potato used was Red King Edward, grown locally.

With the following exceptions, the chemical substances used were all purchased from commercial sources: *Homocatechol*: creosole prepared from vanillin was refluxed with constant boiling HI; the resulting homocatechol was removed by vacuum distillation and purified by crystallization from benzene. *Caffeic acid*: protocatechuic aldehyde prepared from piperonal was refluxed with malonic acid in pyridine with a trace of piperidine as catalyst; the caffeic acid was crystallized from hot water. *p-hydroxycinnamic acid*: *p*-hydroxybenzaldehyde was refluxed with malonic acid in pyridine with a trace of piperidine as catalyst; crystallized from hot water. *Di-sodium*

dihydroxymaleate: prepared from tartaric acid by the method of Neuberg and Schwenk (1915). *Oxalacetic acid*: prepared by the method of Wohl and Oesterlin.

PART I

In an earlier paper Boswell and Whiting (1938) stated that 66 per cent. of the oxygen uptake by slices of potato tuber was controlled by the catechol oxidase system. Baker and Nelson (1943) while criticizing the evidence from which we reached this conclusion agreed that the conclusion was correct and produced supporting evidence. Whatever is the exact interpretation of their curves showing oxygen uptake following the addition of catechol (interpretations other than the one they use are possible), both their curves and ours show the fundamental point quite clearly, namely, that after the catechol oxidase enzyme has been completely inactivated following the addition of catechol the tissue slices still contain a residual respiratory activity of some magnitude. With regard to the conclusion reached by Baker and Nelson that 85 per cent. of the uptake of the tissue slices was controlled by the catechol oxidase system, in contrast to our value of 66 per cent., five years' work has provided abundant evidence that the exact proportion of the uptake dependent upon this oxidase varies with the season of the year and with the variety of potato.

The use by Baker and Nelson of 4-tertiary butyl catechol is of interest, but the observations recorded are of little value and do not elucidate the problem, much less invalidate our conclusions. This substance is described as not inactivating the oxidase isolated from the common mushroom and the assumption is made that it does not inactivate that from the potato. In this case any inhibition of respiration which follows the addition of this catechol derivative to the tissue must be due to its action on some part of the respiratory system other than the oxidase, for example, oxidation may occur between the 4-tert. butyl catechol oxidant and the natural catechol compound present in the tissue, this would destroy the respiratory activity without any inactivation of the enzyme. In addition, there is no evidence to show how much of the butyl catechol is oxidized; until this is known the rapid fall in the uptake following the peak value cannot be correctly interpreted. The results obtained using the butyl catechol are in quite a different category from those obtained using homocatechol, catechol, and 4-nitrocatechol.

Boswell and Whiting used a naturally occurring polyphenol prepared from potato tubers. For further work on the oxidase system this compound is unsuitable since its preparation is laborious and the final product is a gummy mass of indefinite constitution and variable activity. It is improbable, however, that the preparation contained any organic acids since it was an ether-insoluble residue obtained by the extraction of an acid solution. In the search for a polyphenol suitable for use in further work on oxidases in respiration systems a wide range of phenols has been examined and the results are recorded in Table I.

TABLE I

Substance.	Amount (mg.).	Duration of experiment (hours).	Excess O ₂ (μl.). CO ₂ (μl.).		Remarks.
Catechol . . .	2.2	0.5	80	—	Inactivates enzyme.
Protocatechuic acid	0.77	2	86	26	E_0 0.883.
	1.55	2	92	41	
Homocatechol . .	1.0	2	82	—	Inactivates enzyme.
	1.1	2	97.5	—	
Caffeic acid . . .	0.2	7	99.5	60	125 μl. per mg. caf-
	0.22	4	60.3	32	feic acid corres-
	0.44	4	113	81	ponds to 1 mol O ₂
	0.5	7	199	173	per mol caffeic acid.
	0.85	3.5	194	126	
Dihydroxyphenyl-	0.32	4.3	107	56	E_0 0.800.
alanine ('Dopa')	0.57	4.3	93	26	119 μl. O ₂ per mg.
	0.96	3.0	103	68	'dopa' corresponds
	1.06	4.3	140	98	to 1 mol O ₂ per mol
					'dopa'.
Phenol	1.1	2	No effect.		
<i>m</i> -hydroxybenzoic acid	1.1	2	No effect.		
<i>p</i> -hydroxybenzoic acid	1.2	4	No effect.		
<i>p</i> -hydroxy cinnam- ic acid	1.2	4	No effect.		
<i>p</i> -cresol	0.9	4	141	—	Inactivates enzyme.
	1.14	4	288	—	
<i>l</i> -tyrosine	0.75	1.5	No effect.		
	0.93	3.3			
	1.10	3.3			
β -phenyl alanine	1.1	2.0	No effect.		
Tannic acid . . .	1.7	4	70	64	
	3.2	2.3	65	32	
Gallic acid	0.85	4.0	179	137	E_0 0.799.
	1.1	2.7	146	128	
	1.7	2.0	132	114	
Pyrogallol	0.9	2.0	40	19	

The E_0 values are taken from Ball and Chen (1933).

The only monohydroxy-phenol tested which is oxidized by the whole cell is *p*-cresol. This oxidation results in the inactivation of the enzyme as does that of catechol and homocatechol. This is to be expected since the initial oxidation product of *p*-cresol is homocatechol. Fig. 1 illustrates the respiratory activity of potato slices following the addition of *p*-cresol in one case and homocatechol in the other. There is a clear distinction between the oxygen uptake curves. The oxidation of homocatechol inhibits the enzyme activity with great rapidity, the maximum value of uptake reached in the first 10 minutes is followed by a very rapid decline. This is in accordance with the generally held view that the inactivation of the enzyme is due to some oxidation product, cf. the curves following the addition of catechol, Boswell and Whiting (1938). The oxygen uptake curve following the addition of *p*-cresol shows a much lower maximum value than that with homocatechol,

but the value is maintained with little or no decline over a long period, suggesting that the initial phase of *p*-cresol oxidation is to homocatechol and that little or no quinone formation takes place until the *p*-cresol is almost wholly oxidized to homocatechol. The lower maximum is in agreement with observations on the free enzyme oxidations which show that *p*-cresol is oxidized more slowly than homocatechol. The excess uptake from the

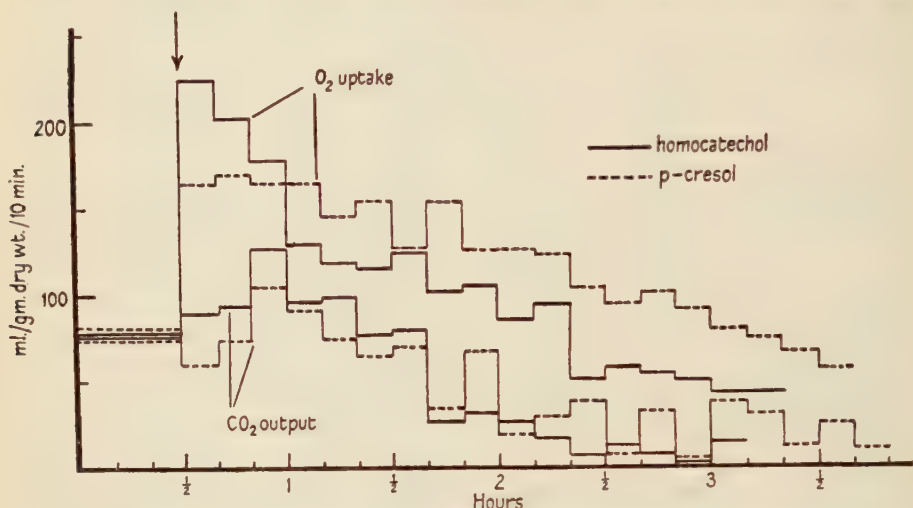


FIG. 1. The effect of the addition of *p*-cresol in one experiment and homocatechol in another on the respiration rate of potato tuber slices.

addition of *p*-cresol to the point at which a continuous decline in the uptake occurs is approximately equal to $\frac{1}{2}\text{O}_2$. Gregg and Nelson (1940) found that when *p*-cresol was oxidized by the free enzyme in the presence of a trace of catechol the cresol was transformed wholly to homocatechol before any quinone was formed. The slight acceleration of the CO_2 output which occurs following the addition of the *p*-cresol to the slices indicates that even in the early stages a trace of quinone is formed; the increase in the CO_2 output following the homocatechol oxidation is much more pronounced.

The mechanism of monohydroxyphenol oxidation has frequently been investigated using *p*-cresol as a substrate and enzyme preparations of varying degrees of purity. One of the points at issue is whether the same enzyme oxidizes both mono- and dihydroxy-phenols or whether two separate enzymes are required. Many of the reported observations are of little value since the enzyme preparations used were very impure. Keilin and Mann (1938) using highly purified preparations of polyphenol oxidase found that the enzyme oxidized both *p*-cresol and tyrosine, the latter very slowly, an interesting observation since the whole cell does not oxidize it at a measurable rate. With increasing purity of enzyme the ratio of the rate of O_2 uptake in catechol oxidation to the rate of uptake in *p*-cresol oxidation and the length of the induction period in *p*-cresol oxidation are increased. The purest enzyme

preparation catalysed *p*-cresol oxidation only when the enzyme was used in very high concentration. It would appear therefore that the enzyme is highly specific for the oxidation of *o*-dihydroxy-phenols and that monophenols require the presence of an additional factor. Onslow and Robinson (1928) have suggested that this factor is a quinone, but this view is opposed by Graubard and Nelson (1935) and by Pugh (1930). It is, however, generally agreed that a trace of catechol accelerates the oxidation of *p*-cresol and removes the induction period observed when enzyme preparations of high purity are used. Consideration of the constitution of polyphenol oxidase, of our knowledge of enzyme action, and of Szent-Györgyi's (1938) theory of catechol oxidation lead to the conclusion that the enzyme only oxidizes *p*-cresol through the formation of a *p*-cresol-*o*-dihydroxyphenol complex which is adsorbed on the protein part of the enzyme, electron exchange between the copper atoms and the phenols leading to the *p*-cresol→homocatechol oxidation. The rapid oxidation of the *p*-cresol by crude preparations of the enzyme and by the whole tissue would therefore be due initially to the formation of a complex between the phenol and the naturally occurring polyphenol; subsequently the homocatechol formed would take part in complex formation. The decreasing rate of *p*-cresol oxidation with increasing purification of the enzyme can be ascribed to the progressive removal of the natural polyphenol adsorbed on the enzyme protein. The capacity of catechol to accelerate the oxidation of *p*-cresol would therefore be due to its ability to form part of the essential complex.

The slow oxidation of tyrosine by the free enzyme and the absence of any recognizable rate of oxidation by the whole cell suggest that tyrosine does not enter into a complex with the *o*-dihydroxyphenols as readily as does *p*-cresol. The low rate of oxidation would reflect the small concentration of the complex which contains tyrosine. All the available evidence is against the view that the low solubility of tyrosine is the limiting factor.

The inactivation of oxidase activity following the oxidation of dihydroxy-phenols is, from Table I, limited to a small number of these compounds. This raises the question of the mechanism of the inactivation. It is established that the oxidation of catechol requires one molecule of oxygen for each molecule of catechol oxidized and that little, if any, H_2O_2 is formed. From the work of Jackson (1939) it is clear that Wagreich and Nelson's view that catechol is oxidized to a hydroxyquinone is incorrect; the initial oxidation product is benzoquinone. The second atom of oxygen is used in secondary oxidations following the formation of the quinone, see Wagreich and Nelson (1938). Ludwig and Nelson (1939) have stated that benzoquinone does not inactivate the enzyme and that the inactivation during the oxidation of catechol involves a constant oxygen uptake per unit of enzyme activity and per unit of copper content. It would appear therefore that the inactivation of the enzyme is due to the structure of the secondary oxidation products of catechol. The inactivators, catechol, homocatechol, *p*-cresol, and 4-nitrocatechol, are all closely related to one another structurally, and it is possible

that the power of enzyme inactivation is related to the redox potentials of their respective phenol \rightleftharpoons quinone systems. Pugh and Raper (1927) and Richter (1934) have recorded observations which show that the activity of catechol oxidase is much affected by the presence of substances which reduce the potential of the system. When those dihydroxyphenols which do not inactivate the enzyme are considered, using the data of Ball and Chen, it is

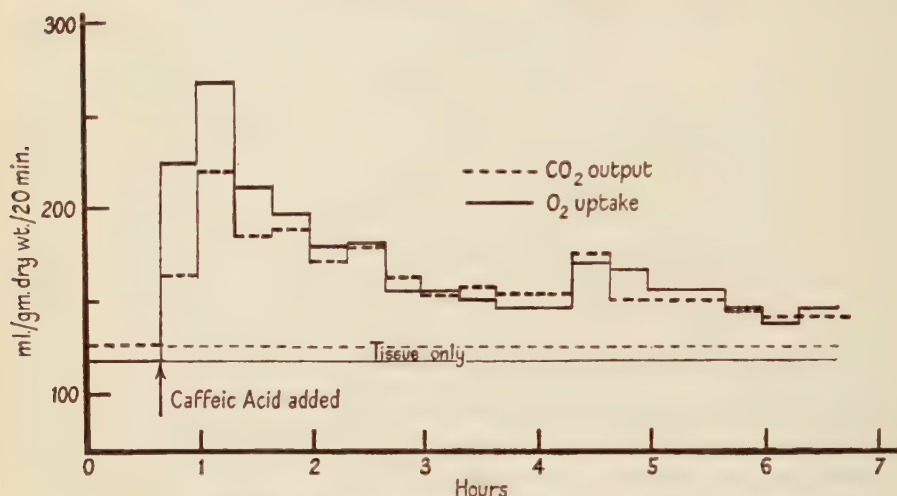


FIG. 2. The effect of the addition of caffeic acid on the respiration rate of potato-tuber slices.

clear that the explanation of the inactivation on the basis of redox potentials must be discarded. Catechol, an inactivator, and 'dopa' and gallic acid, non-inactivators, have E_0 values very close together, while protocatechuic acid, with a potential 91 mv. in excess of that of catechol, and pyrogallol, with a potential 79 mv. below that of catechol, are both non-inactivators. The explanation of the difference between these two groups of substances in their effect on catechol oxidase must be sought in the molecular structure of their secondary oxidation products. On this view the enzyme is inactivated because the oxidants are of such a structure that either through their adsorption on the enzyme protein the formation of certain links between the phenol and the enzyme essential to phenol oxidation is prevented, or the copper atoms are bound in complexes and thereby eliminated from the enzyme. The observation of Adams and Nelson (1938) that the enzyme during catechol oxidation can be protected from inactivation by the addition of a protein, e.g. gelatin, to the reaction medium supports the view that the inactivation is due to the adsorption of the secondary oxidation products on the enzyme protein. The amount of gelatin added is so many times greater than the amount of enzyme protein present that the catechol oxidants are adsorbed mainly by the gelatin, and the enzyme remains active. The non-inactivators are those substances whose secondary oxidation products are of such a structure that either they

are not adsorbed on the enzyme protein or if adsorbed do not prevent phenol oxidation.

Fig. 2 gives the O_2 uptake and the CO_2 output of tissue, following the addition of caffeic acid. The curves are comparable with those obtained by Boswell and Whiting using the natural polyphenol present in potato tubers. An excess O_2 uptake after 7 hours equivalent to 4 mols O_2 for each mol of caffeic acid present, and the apparently permanent increase of both O_2 uptake and of CO_2 output following the addition of caffeic acid, suggest that the caffeic acid takes part in a cyclic system being alternatively oxidized and reduced, in the first reaction O_2 being absorbed and in the second CO_2 being evolved. The complete inhibition of excess CO_2 output when caffeic acid is added to tissue the respiratory activity of which has been partially inhibited by a prior addition of malachite green shows that the CO_2 output follows an oxidation involving a dehydrase. The reduction of the caffeic acid quinone by some H-donor present in the tissue thus completing the redox system would be a reaction involving a dehydrase. It would appear therefore that the caffeic acid forms part of a system identical with that proposed by Boswell and Whiting for the natural polyphenol in the potato tuber. The results of prolonged experiments using 'dopa' and gallic acid suggest that these substances can also enter into similar cyclic systems. Certain observations on the nature of the hydrogen donor are recorded in part IV.

It has been observed (Table II) that the length of time during which the slices are washed prior to use modifies the O_2 uptake and CO_2 output curves following the addition of caffeic acid.

TABLE II

Oxygen Uptake and Carbon Dioxide Output (μ l. per gm. dry wt. per 10 min.)

	O_2	CO_2	R.Q. (mean value).
Tissue only	61	60	0.98 3rd-5th day incl.
1.0 mg. caffeic acid added.			
After 2 days' washing	88	68	0.78 during first 90 min.
" 3 "	98	96	0.98 " " "
" 4 "	106	90	0.94 " " "

The data suggest that the supply of H-donor is an important factor in determining the curve forms, particularly in the early stages.

In Table III are set out observations on the oxidation of catechol by tissue

TABLE III

Oxygen Uptake (μ l.) during the initial 10 mins. following the Addition of Catechol

Tissue alone.	Tissue + sodium azide (0.5 ml. M/20).	Tissue + malachite green (0.5 ml. 1/500).
41.7	5.3	—
41.1	5.1	—
71.2	—	61.2
78.6	—	71.6

slices following the partial inhibition of their respiratory rate by certain substances. Since sodium azide is recognized as an inhibitor of oxidases and malachite green as an inhibitor of dehydrases it is clear that the oxidation of catechol does not involve the intermediate operation of a dehydrase. The slight reduction of O_2 uptake following the addition of catechol to tissue+malachite green shows that the inhibitor prevents the reduction of the quinone to catechol and therefore reduces the amount of catechol available for oxidation.

It was noted by Boswell and Whiting that acetate buffer solutions had a depressor effect upon the rate of respiration of potato slices. Table IV shows respiration rates in phosphate and acetate buffers, pH 5.5.

TABLE IV

Gas Intake and Output (μ l. per gm. dry wt. per hr.)

	O_2	CO_2
Phosphate buffer	444	440
Acetate „	382	401

The values set out in Table V show that the inhibiting effect of acetate is at least in part an inhibition of catechol oxidase.

TABLE V

Excess O_2 Uptake (μ l.) during initial 30 mins. following the Addition of 0.3 ml. M/25 Catechol

Tissue in phosphate buffer, pH 5.5	130	113
Tissue in phosphate buffer, pH 5.5+0.5 ml. M/5 acetate	70.5	70.2

The specificity of acetate as an enzyme inhibitor has not been determined.

PART II

Szent-Györgyi and his co-workers (1938) have postulated the existence of a third, major, direct oxidase system in plant tissues, one which would utilize dihydroxymaleic acid as a substrate, analogous to catechol and its oxidase. They claim to have isolated from a number of plant tissues, including potato tuber, dihydroxymaleic acid oxidase in a crude state. The existence of this enzyme has been questioned by Swedin and Theorell (1940), who consider that there is no basis for the assumption that this oxidase and peroxidase are separate enzymes. A major difficulty lies in the fact that there is little evidence that dihydroxymaleic acid exists in plant tissues and it has never been isolated from this source. The acid is unstable in aqueous solution and decomposes on warming at $50^\circ C.$ to give CO_2 and glycollic aldehyde.

The addition of disodium dihydroxymaleate to slices of potato tuber results in increases in the rates of both O_2 uptake and CO_2 output but the increments are not permanent, Fig. 3. In Table VI are set out a number of results showing O_2 uptake and CO_2 output; in each case the experiment proceeded until both O_2 and CO_2 rates had fallen to the pre-addition values.

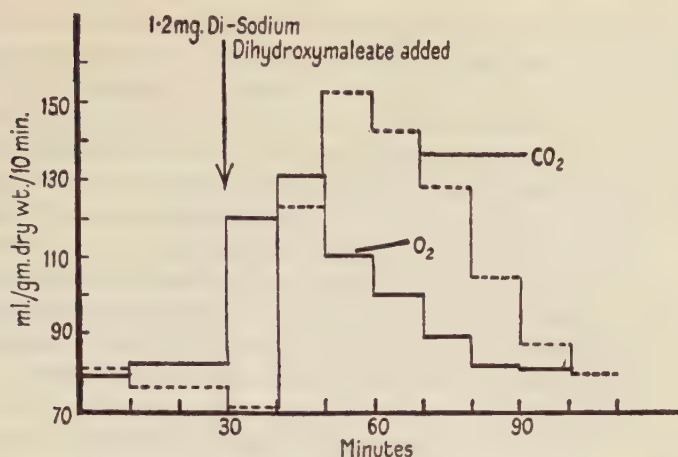


FIG. 3. The effect of the addition of disodium dihydroxymaleate on the respiration rate of potato-tuber slices.

TABLE VI

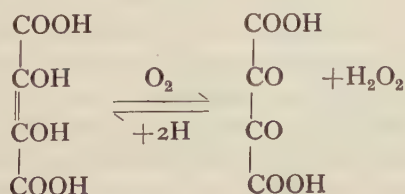
Excess Uptake and Output (μ l.)

Wt. of disodium dihydroxymaleate added (mg.).	O ₂	CO ₂
0.32	19	69 (28.4+40.6)
0.68	31	105 (50.3+54.5)
1.00	35	109 (51.5+57.5)
1.00	36	146 (70.0+76.0)
1.32	44	132 (64.0+68.0)

Under the heading 'Excess CO₂' the figures in brackets show the way in which the total is made up, the first figure is the amount of CO₂ evolved as gas, while the second is the increase in the amount of CO₂ bound during the experiment.

It appears that with increasing quantities of disodium dihydroxymaleate there are increases in both excess O₂ uptake and CO₂ output, but no obvious quantitative relationship exists between these three values. The limitation of O₂ uptake is not due to any inhibition of the enzymes concerned by the oxidation product as occurs in the case of catechol oxidase and catechol.

The graphs in Fig. 3 do not support the view that the added acid forms part of a redox system of the following type:



When this reaction occurs the graphs assume the form shown for caffeic acid (Fig. 2). It may be objected that this graph form is only obtained when the H-donator which reduces the oxidant and thereby completes the redox system is produced at a rate greater than that at which the cells normally use it. Banga and Szent-Györgyi (1938) from the use of redox indicators have concluded that the potential of the dihydroxymaleic acid \rightleftharpoons diketo system is of the same order as that of the ascorbic acid \rightleftharpoons dehydroascorbic acid system, and since in the potato tuber slices ascorbic acid and not the oxidant is always found, it is to be expected that the dihydroxymaleic acid would be present rather than its oxidant; this could only occur if there were present in the cell excess of some substance which acted as H-donator to the oxidant. It may be expected, therefore, that if dihydroxymaleic acid and its oxidase, or even peroxidase with H_2O_2 , form a redox system in the tissue the curve following the addition of the acid or its salts to the tissue should have a form similar to that of Fig. 2, since excess H-donator would always be present in the tissue, instead of the form which is recorded in Fig. 3. That figure does not therefore support the view that dihydroxymaleic acid forms part of a redox system in the tissue. Further the excess O_2 uptake is never great enough to be equivalent to 1 mol O_2 per mol acid added, and values greater than this must be reached to establish the existence of a cyclic redox system.

The nature of the reaction which the dihydroxymaleic acid undergoes in the tissue can be outlined from the CO_2 values in Table VI. Since during the experiments there were considerable increases in the basicity of tissue + medium, as shown by the increase in the CO_2 bound, it may be considered that the disodium dihydroxymaleate was decomposed by decarboxylation with liberation of sodium ions. In support of this view it may be noted that when the free acid was used in place of the salt the CO_2 output was of the same order as with the salt, but instead of part being evolved as gas and part bound in the tissue it was almost wholly evolved as gas. If decarboxylation were part of the total oxidative decomposition of the acid the R.Q. would be 2; since the value is always greater than 2, the reaction must involve only partial oxidation leaving an organic residue. Neuberg and Schwenk (1915) observed the decarboxylation of this acid by yeast and yeast juice.

Since the O_2 uptake is the same when the sodium salt is added to the tissue in the presence or absence of malachite green, it may be concluded that the O_2 uptake is due to an oxidase direct or indirect without the intermediate link of a dehydrogenase, or the reaction is not enzymic. It is of interest that Swedin and Theorell (1940) have shown that peroxidase and dihydroxymaleic acid produce the necessary H_2O_2 spontaneously, the enzymic iron stimulating the autoxidation of the acid with the formation of H_2O_2 .

Sodium azide reduces the excess O_2 uptake; the extent of the reduction varies considerably, but in no case has it exceeded 50 per cent. This may indicate that the enzyme is relatively resistant to azide, but more probably that in the presence of azide it is oxidized by a non-enzymic system; it is already known that autoxidation in the presence of iron can proceed at a high

rate. The azide will, however, have a limiting effect, the H_2O_2 formed through autoxidation being unused in further oxidations since the necessary peroxidase is inactivated by azide. The excess CO_2 output was not reduced by the presence of azide, which shows that the CO_2 output was in no way connected with the oxidase; the output was, however, reduced by about 30 per cent. in the presence of malachite green. Part of the CO_2 output would therefore appear to result from a reaction preceded by a dehydrogenation. Since the dye has no effect on the O_2 uptake, this dehydrogenation is not associated with any part of the excess O_2 uptake.

It would appear, therefore, that dihydroxymaleic acid added to tissue does not enter a redox system, that its oxidation is in part enzymic, and that the CO_2 output is the product of at least two separate reactions, one of which is associated with an oxidation.

PART III

The potato tuber contains ascorbic acid but does not contain a direct oxidase capable of oxidizing it in the presence of air (Johnson and Zilva, 1937). Stone (1937) mistook the oxidation of ascorbic acid by the phenolase+phenol in the potato tuber extract for the action of a direct oxidase. In the whole potato tuber the quantity of ascorbic acid and the ratio between ascorbic acid and dehydroascorbic acid vary with the duration of storage. Steward and Preston (1940) state that in washed potato tuber slices the ascorbic acid is wholly in the reduced condition. Records made during the course of this work confirm their observation.

When ascorbic acid, dissolved in 0.02 M phosphate buffer (pH 5.5) made up with glass-distilled water, was added to washed slices of potato tuber the rate of O_2 uptake increased rapidly to a maximum value and then fell away to the pre-addition value after about one hour. The CO_2 output was first stimulated and then depressed (Fig. 4). The excess O_2 uptake was of the same order as the value calculated on the basis of 0.5 mol O_2 uptake for each molecule of ascorbic acid oxidized (Table VII).

TABLE VII

Ascorbic acid added (mg.).	O_2 uptake ($\mu\text{l.}$).	Calculated O_2 uptake ($\mu\text{l.}$).
2.02	112	127
1.96	126	123
0.98	51	62

Analysis of the medium and tissue following the completion of the O_2 uptake showed that only a small fraction of the ascorbic acid either entered the tissue or was retained by it. The greater part of the acid was present in the external medium in the oxidized state (Table VIII). The acid present in the tissue was in the reduced form.

TABLE VIII

Ascorbic acid added (mg.).	Dehydroascorbic acid in external medium (mg.).	Ascorbic acid in tissue less estimated original content (mg.).
1.15	1.09	0.12
1.08	1.03	0.11

Making allowance for the initial ascorbic acid content of the tissue, it appears that the amount of ascorbic acid present in the tissue at the end of the oxidation period was less than 11 per cent. of the amount added. The ascorbic acid was extracted from the tissue by grinding with 8 per cent. acetic acid; the rapid autoxidation noted by Steward and Preston was not observed. The ascorbic acid was titrated against 2.6 dichlorophenolindophenol. The dehydroascorbic acid was reduced with H_2S overnight, the excess being removed in a stream of hydrogen.

The total excess O_2 uptake was not reduced in the presence of either sodium azide or malachite green, nor was the excess O_2 uptake prolonged at a lower rate over a time interval greater than when the ascorbic acid was added to the tissue alone. In the presence of malachite green the excess CO_2 output in the first phase after the addition of the ascorbic acid (stimulation phase) was reduced to about half the value obtained in the absence of the inhibitor. The second phase (depression phase) could not be observed since the CO_2 output from the tissue in the presence of 1/5,000 malachite green was only 10 per cent. of the value in the absence of the inhibitor. In the presence of sodium azide the excess CO_2 output in the first phase was greatly stimulated, being 2–3 times greater than that obtained when the ascorbic acid is added to the tissue alone. The second phase, depression, was not present since the level of the CO_2 output in the presence of M/200 sodium azide was about 30 per cent. of the value in the absence of the inhibitor.

Certain observations were made using a solution containing dehydroascorbic acid, prepared by dissolving ascorbic acid in distilled water from a metal still. Potassium hydrogen phosphates were added to give a 0.02 M solution at pH 5.5. The solution was shaken in air until titration of a sample showed that more than 90 per cent. was oxidized. When this solution was added to slices of potato tuber the O_2 uptake increased slightly as the residual ascorbic acid was oxidized. The CO_2 output showed changes almost identical with those obtained following the addition of ascorbic acid; with quantities below 0.6 mg. there was no initial stimulation, only a considerable depression of the rate, while with quantities above 0.6 mg. an initial stimulation and subsequent depression occurred comparable with those in Fig. 4.

The fact that the total excess O_2 uptake was approximately equal to the value calculated on the basis of $\frac{1}{2}$ mol O_2 per mol ascorbic acid oxidized and that 90 per cent. of the added ascorbic acid was recovered from the external medium as dehydroascorbic acid suggest either that the oxidation occurred on the surface of the tissue or that the acid entered the cells and was oxidized

and excreted immediately into the outer medium without taking part in any other reactions within the cells. Since the oxidation is insensitive to both sodium azide and malachite green and is therefore non-enzymic and metal catalysed, it is more probable that the oxidation occurred on the surface of

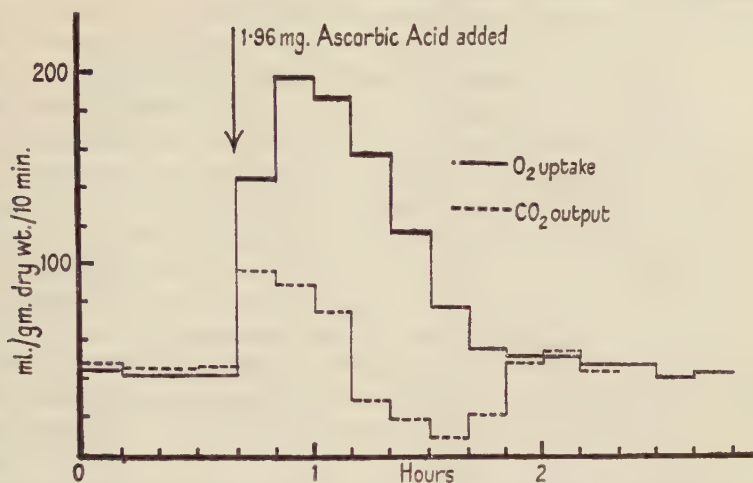


FIG. 4. The effect of the addition of ascorbic acid on the respiration rate of potato-tuber slices.

the tissue, may be on the cellulose walls, without the ascorbic acid entering the cells.

The small amount of added ascorbic acid which was present in the tissue at the end of the oxidation probably penetrated as dehydroascorbic acid since the CO_2 output curve has the same shape when dehydroascorbic acid was added to the tissue instead of ascorbic acid. The oxidized acid is reduced in the tissue. It is not known whether the acid becomes involved in a cyclic redox system or whether the first reduction is final, but if the first alternative occurs there is no corresponding O_2 uptake. The reaction or reactions in which the acid is involved are reflected in the CO_2 output which appears to be the resultant of opposing reactions. With quantities of ascorbic acid in excess of 0.5 mg. the output of CO_2 is initially stimulated and subsequently depressed below the 'tissue-only' level, returning to this value after some time (Fig. 4). With added quantities of ascorbic acid less than 0.5 mg. there is no initial stimulation, only the penultimate depression. Competition would therefore appear to occur between two systems for the dehydroascorbic acid. From the work of Ball and Chen (1933) on the potentials within plant tissue it is to be expected that an excess of H-donators is present in the cells; further, this view is supported by the observation that ascorbic acid and not its oxidation form is always present in tissue slices. It would be reasonable, therefore, to suppose that the initial increase of the CO_2 output is due to a reaction between dehydroascorbic acid and the accumulation of a suitable H-donor in the tissue. The observation that sodium azide increases the excess CO_2

output in the initial phase is in accord with this explanation of the origin of the excess CO_2 output, since in the presence of sodium azide the oxidase systems of normal respiration are inhibited and H-donators will accumulate to an even greater extent than in normal tissue. In opposition to the increased output of CO_2 following the addition of ascorbic acid is the reaction which results in a decrease in the CO_2 output. With small additions of ascorbic acid (< 0.5 mg.) the depressor reaction dominates the stimulation reaction; with larger amounts the stimulating effect is dominant during the early stages when both dehydroascorbic acid and the H-donator are abundant, but as these decrease in quantity the depressor reaction becomes dominant. This depressor phase in CO_2 output reflects a reaction between dehydroascorbic acid and some system which forms part of the normal respiration process. This reaction does not result in any CO_2 output and the depression in the rate of the output may be as much as 50 per cent. of the 'tissue-only' value. This inhibition must involve a reaction with some part of the polyphenol oxidase system since this is concerned with at least 66 per cent. of the total CO_2 output. That the inhibition is due to a reaction with dehydroascorbic acid and not ascorbic acid is shown by the observation that the same depression in CO_2 output occurs following the addition of the dehydroascorbic acid as with its reductant.

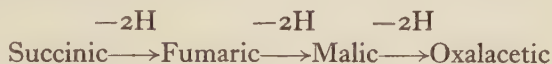
PART IV

The role of organic acids in plant respiration is becoming increasingly clear, particularly the possibility that they are the link between carbohydrate and protein metabolism. Improved analytical methods have made it possible to estimate accurately the small quantities of organic acids present in the tissues of the higher plants. The technique of Thunberg has led to the identification of dehydrogenases capable of oxidizing citric, malic, and succinic acids and to the recognition that these enzyme systems are widespread in the plant kingdom. It was decided, therefore, to investigate the effects of a number of these acids on the respiration rate of sliced tissue as a preliminary to further experiments for determining the precise nature of the metabolic products which result. In Table IX are set out the excess O_2 uptake and CO_2 output values for a number of acids. The values recorded were calculated from the differences between the 'tissue-only' rates and the rates which follow the addition of the acid. Each experiment lasted 2 hours and the values in the table are the excess gaseous exchanges over that period. Glycine, glutamic, and aspartic acids had no effect on either the O_2 uptake or the CO_2 output. With comparable experimental conditions seasonal variations in the effects of the other acids were prominent. No information is available as to what extent these acids are oxidized by dehydrogenation without any corresponding O_2 uptake. Further, the values are only apparent since a reduction in the 'tissue-only' rate of respiration due to the presence of some or all of the acids would pass unrecognized, with the result that the recorded values would not show the full effect of the acids.

TABLE IX

Experiment No.	Substance.	Amount added (mg.).	Excess (μ l.).	
			O ₂	CO ₂
3.13	Succinic acid	4.8	53	98
3.17	" "	4.8	50	94
1.6	" "	1.9	26	79
3.13	Fumaric acid	4.8	18	51
6.2	" "	2.0	16	32
6.2	" "	2.0	18	36
1.6	" "	1.8	13	40
3.7	l-malic acid	2.0	11	41
1.6	" "	2.2	15	47
3.30	Oxalacetic acid	1.0	7	74
3.30	" "	1.0	11	71
1.19	" "	1.7	15	66
1.7	Pyruvic acid	2.3	33	73
5.31	" "	1.9	57	83
3.11	Citric acid	6.8	10	52
3.7	" "	2.6	4	58
3.7	" "	2.6	15	59
3.7	Lactic acid	2.5	5	42
3.7	" "	2.5	4	38

The oxidation of succinic, fumaric, and malic acids suggests that the following relationships may hold:



These oxidations should be inhibited by malachite green since they involve dehydrogenases. It has been observed that when succinic, fumaric, and malic acids are added to tissues the respiration rates of which have been reduced by this dye, no excess uptake of O₂ or output of CO₂ results.

The decomposition of oxalacetic acid may be expected to follow this equation



The observation that the output of CO₂ is only very slightly reduced in the presence of malachite green supports the view that the decomposition of oxalacetic acid is a decarboxylation unaccompanied by oxidation. This reaction may not be enzymic since oxalacetic acid is unstable and is very rapidly decomposed in the presence of proteins. The small O₂ uptake which accompanies the CO₂ output may arise from the oxidation of the resulting pyruvic acid since pyruvic acid is very readily oxidized by the tissue. The resultants of the pyruvic acid oxidation are unknown, but the observation that the output of CO₂ is only partially reduced in the presence of malachite green suggests that decarboxylation unaccompanied by oxidation plays a major part.

Comparison of the CO₂ outputs following the additions of succinic, fumaric, and malic acids (expt. 1.6), assuming that the tissue absorbs the acids in

approximately equal quantities, shows that part of the CO_2 output following the addition of succinic acid must be from some reaction catalysed by the succinic \rightarrow fumaric oxidation, since the CO_2 output following the addition of succinic acid is much in excess of that from both fumaric and malic acids. The other part of the CO_2 output will be derived from the fumaric, malic, oxalacetic chain with the additional possibility that some comes from a reaction catalysed by the malic \rightarrow oxalacetic oxidation.

The close agreement between the excess O_2 and CO_2 values following the additions of fumaric and malic acids shows the presence of an active fumarase.

Both citric and lactic acids give rise to considerable outputs of CO_2 with only small increases in the rate of O_2 uptake. Since in the presence of malachite green the excess CO_2 is reduced to a small value, it may be concluded that the production of CO_2 is either preceded by or associated with an oxidation involving a dehydrogenase and that no corresponding uptake of O_2 occurs under the experimental conditions.

Observations were made of the effect on the respiration rate of adding an organic acid and caffeic acid successively to the same tissue in comparison with the effect of adding caffeic acid alone. The organic acid was added before the caffeic acid in those experiments in which the tissue was treated with both acids, since if the caffeic acid were added first the excess quinone which is formed would be rapidly removed as the result of secondary oxidations which occur between the quinone and the caffeic acid. By adding the caffeic acid after the organic acid the quinone is formed in the presence of the organic acid with which it may react. The acids used were succinic, fumaric, malic, oxalacetic, pyruvic, citric, aspartic, glutamic, and glycine. The results fall into two groups (Table X). When caffeic acid is added to a tissue which has been previously treated with an amino-acid the excess O_2 uptake and CO_2 output following the addition of the caffeic acid are greater than the values obtained when the caffeic acid is added to the untreated tissue. Fig. 5 illustrates the effect of adding glutamic acid and caffeic acid to tissue and the effect of adding caffeic acid alone. The particular experimental results illustrated were used since owing to the form of the CO_2 output curve following the addition of the caffeic acid alone all the curves are well separated from one another. The form of this CO_2 output curve is dependent upon the length of time during which the tissue was washed prior to use, and this explains the difference in form between the CO_2 output curves following the addition of caffeic acid illustrated in Figs. 2 and 5. However, Fig. 5 is only an extreme example of the influence of amino-acids on the excess O_2 uptake and CO_2 output following the addition of caffeic acid, and does not differ fundamentally from the curves obtained using this and other amino-acids on other occasions.

The addition of non-nitrogen-containing organic acids gave most interesting results. In the presence of these acids the addition of caffeic acid results in much smaller excess O_2 uptake and CO_2 output values in comparison with the values obtained when caffeic acid is added alone. In other words, these

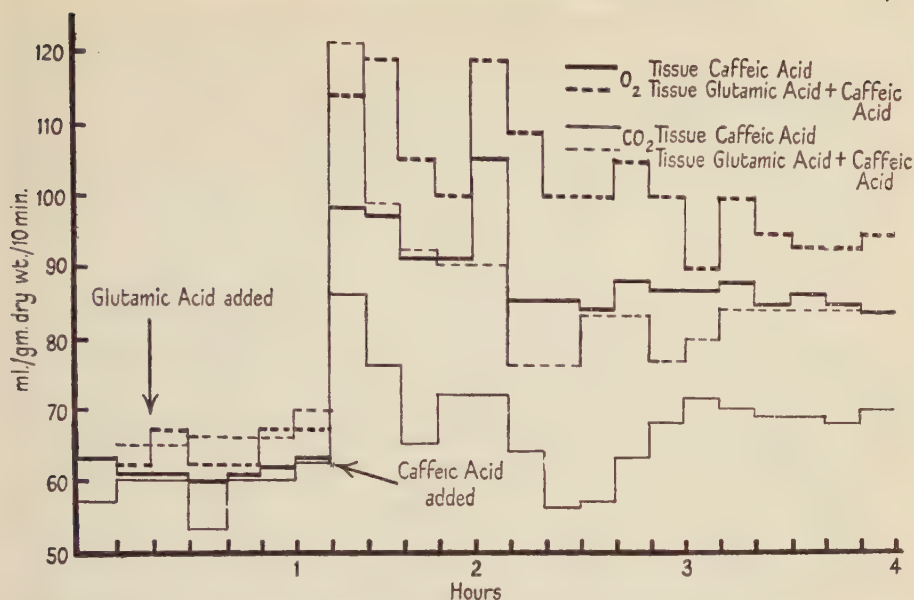


FIG. 5. To illustrate the effect which a prior addition of glutamic acid has on the increase in the rate of respiration which follows the addition of caffeic acid.

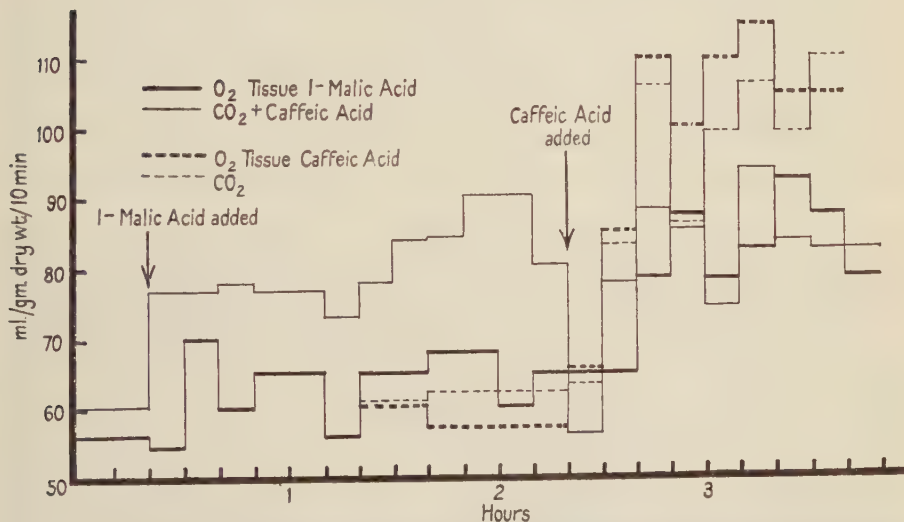


FIG. 6. To illustrate the effect which a prior addition of l-malic acid has on the increase in the rate of respiration which follows the addition of caffeic acid.

acids appear to depress the rate of oxidation of caffeic acid and the consequent output of CO_2 . Fig. 6 illustrates the effect of adding caffeic acid to the tissue alone and that following an earlier addition of l-malic acid.

The results from two experiments are recorded below in Table X; each experiment is divided into three phases (cf. Fig. 6). In the first phase the

respiration rate of the tissue alone is measured; in the second the rate is measured after the addition of the organic acid under investigation, and during the third phase the rate is measured after the addition of caffeic acid. For comparison the rate of respiration following the addition of caffeic acid alone is also recorded. The O_2 and CO_2 values are stated as $\mu l.$ per gm. dry wt. per 10 min. The values for phases 2 and 3 are average values over periods of 2 hours.

TABLE X

Experiment No.		61.44					2312.43		
Acids added.		Caffeic acid alone.	Pyruvic acid.	Malic acid.	Succinic acid.	Fumaric acid.	Caffeic acid alone.	Glutamic acid.	Aspartic acid.
Phase 1. Tissue only.	O_2	57	63	56	58	62	66	67	65
	CO_2	61	65	60	59	61	60	62	62
Phase 2. Tissue + organic acid.	O_2	—	79	62	68	64	—	70	69
	CO_2	—	95	80	92	75	—	67	62
Phase 3. Tissue + organic and caffeic acids.	O_2	—	96	80	88	80	—	125	122
	CO_2	—	100	80	96	83	—	101	103
Phase 3a. Tissue + caffeic acid.	O_2	98	—	—	—	—	114	—	—
	CO_2	96	—	—	—	—	73	—	—
2/1	O_2	—	1.25	1.11	1.17	1.04	—	1.04	1.06
	CO_2	—	1.46	1.33	1.56	1.23	—	1.08	1.00
3/1	O_2	—	1.52	1.43	1.52	1.29	—	1.86	1.88
	CO_2	—	1.54	1.33	1.63	1.36	—	1.63	1.66
3/2	O_2	1.72	1.21	1.29	1.30	1.25	1.73	1.79	1.77
	CO_2	1.57	1.05	1.00	1.04	1.11	1.22	1.51	1.66

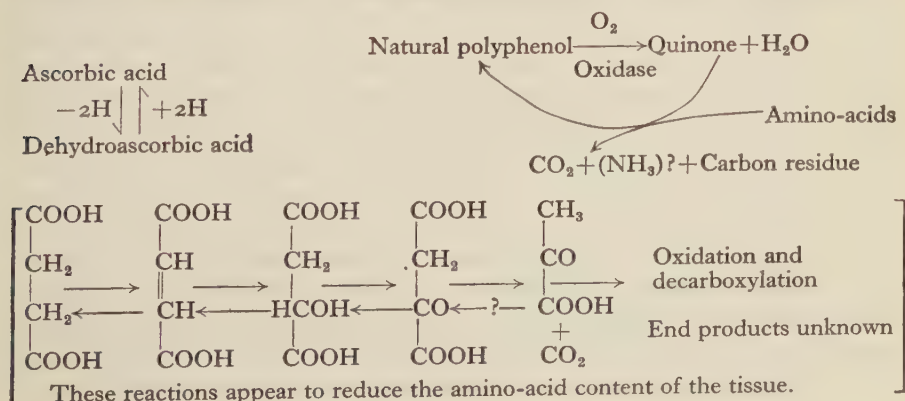
The experiments of Robinson and McCance (1925), Happold and Raper (1925) have shown that the free polyphenol oxidase in the presence of a suitable phenol is capable of oxidizing certain amino-acids, including leucine and glycine, with the liberation of ammonia and CO_2 . Hubbard (1938) when confirming these observations has shown that the phenol forms a reversible phenol $\xrightleftharpoons[-2H]{+2H}$ quinone system. The phenols used successfully were *p*-cresol, catechol, and protocatechuic acid. The system enzyme + tyrosine does not deaminate glycine. *In vitro* therefore the polyphenol oxidase system acts as an amino-acid oxidase mechanism. The experiments described above were designed to determine whether or not the oxidase operated in a similar manner in the whole cell and if amino-acids therefore were the only substances which could act as H-donators in the polyphenol \rightleftharpoons quinone cycle. Indirect evidence is already available that the polyphenol oxidase is part of the mechanism of nitrogen metabolism, since Steward, Stout, and Preston (1940), from their work on the mechanism of salt uptake, consider that it is highly significant that in slices of potato tuber the proportion of the total O_2 uptake dependent upon the polyphenol oxidase system is of the same order as that component which is intimately concerned with protein synthesis and salt accumulation. That the results recorded above establish the position of polyphenol oxidase in nitrogen metabolism is clear from the following argument. The O_2 uptake and CO_2 output from washed slices of potato tuber are dependent upon more than one oxidase system. That part of the gaseous exchanges which is linked with the polyphenol oxidase is limited by the

amount of the natural polyphenol present in the cells (Boswell and Whiting, 1938). By the addition to the tissue of either the natural polyphenol or a substance such as caffeic acid the rate of respiration is permanently increased and when excess is added the supply of the H-donor which reduces the quinone in the redox system becomes the limiting factor. Therefore using the system tissue+caffeic acid it is possible to distinguish between those substances which can reduce the quinone and those which cannot, since the former group by increasing the rate at which quinone is reduced will increase the rate of respiration while the latter group will have no such effect. Only the amino-acids increased the O_2 uptake and CO_2 output following the addition of caffeic acid above the values obtained when caffeic acid was added to the tissue alone. It would appear, therefore, that of the substances tested only the amino-acids were capable of reducing the quinone and thereby increasing the rates of both O_2 uptake and CO_2 output. Polyphenol oxidase would therefore appear to act as an amino-acid oxidation mechanism both in the whole cell and as a free enzyme.

The values recorded in Table X show clearly that the non-nitrogen-containing organic acids which were used had the effect of reducing substantially the excess O_2 uptake and CO_2 output following the addition of caffeic acid, in comparison with the values obtained when caffeic acid was added to the tissue alone. Since the inhibition of the O_2 uptake was less than that of the CO_2 output, it may be concluded that the organic acids did not inhibit the oxidation of the caffeic acid but reduced the amount of H-donor (amino-acid) available to react with the quinone. Such a reduction would be directly reflected in the reduction of the excess CO_2 output and indirectly in the lower excess O_2 uptake, since less of the reduced quinone (caffeic acid) would be available for reoxidation.

DISCUSSION

The detailed discussion of the results has been set forth in the separate parts of the paper. In this section it is proposed to consider the results in relation to the whole problem of the respiration of slices of potato tuber. As a basis for the discussion the results are summarized in the following scheme:



The polyphenol oxidase system is dominant in the respiration of slices of potato tuber. Amino-acids appear to be the H-donators which reduce the quinone and maintain the cyclic redox system. Therefore the major part of the gaseous exchanges involved in the respiration of these slices is linked with their N-metabolism. The mechanism by which the amino-acids are produced within the tissue has not been investigated. The observation that an excess of the H-donator to the quinone is normally present in the tissue and the record that the tissue contains amino-acids are in agreement with the observation that added amino-acids act as H-donators. These observations are in agreement with the conclusions reached by Steward on the relationship between respiration, protein synthesis, and salt absorption.

Since the tissue contains certain four-carbon acids and it has been shown that these form part of the respiratory processes in reactions involving O_2 uptake and CO_2 output, it may be concluded that part at least of the gaseous exchanges which are not controlled by polyphenol oxidase are associated with reactions involving the 3 and 4 carbon acids. Addition of these acids to sliced tissue is followed by increases in the uptake of O_2 and output of CO_2 . The R.Q.s are of little value since the exact nature of the products of metabolism is unknown. In particular the O_2 uptake values may be deceptive since oxidations may occur which are not reflected in any increase in O_2 uptake. This would be a considerable source of error when the amount of the acid added to the external medium is only a fraction of the amount of the same or related acids present in the tissue. Since it is clear that in the presence of added organic acids the amount of amino-acids available as H-donators to the quinone formed by the oxidation of the caffeic acid is reduced, it is possible that the addition of the organic acids reduces the rate of the respiration phase controlled by the polyphenol oxidase system. The excess O_2 uptake and CO_2 output values following the addition of the organic acids would then be less than the real values. The nature of the linkage between the metabolism of organic acids and the supply of amino-acids remains to be investigated. Similarly the relationship between the triose products of glycolysis and protein metabolism has not been elucidated.

Ascorbic acid oxidase is not present in the potato tuber and the oxidation of the acid when added to the external medium appears to be a surface reaction and non-enzymic. The dehydroascorbic acid which penetrates into the tissue reacts with some H-donators: in one reaction directly or indirectly an excess output of CO_2 occurs, while in another reaction the normal respiratory output of CO_2 is inhibited. Since glutathione is present in the potato tuber the dehydroascorbic acid may be directly reduced by this substance and the changes in the rate of CO_2 output may reflect reactions in which the oxidized glutathione is involved. Since dehydroascorbic acid while absent from the slices of tuber is present in the whole tuber after certain periods of storage, it must be concluded that a system capable of oxidizing ascorbic acid exists in the tissue. These observations are compatible with the view that ascorbic acid is the co-enzyme of a redox system, being oxidized by an

H-acceptor produced at a limited rate by the sliced tissue, while the dehydroascorbic acid is reduced by an H-donor present in considerable excess. In the whole tuber the presence of dehydroascorbic acid would reflect changes in the rates of production of the H-donor and H-acceptor leading to a condition where the supply of H-donor is not in excess.

While the addition of dihydroxymaleic acid stimulates the O_2 uptake there are no grounds for supporting Szent-Györgyi's view that dihydroxymaleic acid oxidase constitutes a third major oxidase system in plant tissue.

SUMMARY

1. Four oxidation systems were examined in the potato tuber, namely those involving polyphenols, ascorbic acid, dihydroxymaleic acid, and the four-carbon organic acids.

2. Dihydroxyphenylalanine, caffeic, and gallic acids form cyclic redox systems with the oxidase present in potato tissue.

3. Ascorbic acid may be the co-enzyme of a redox system not directly involving oxygen.

4. No evidence was obtained of the existence of a dihydroxymaleic acid oxidase forming a cyclic redox system comparable with the polyphenol system.

5. The four-carbon acids form part of an oxidation system not controlled by the polyphenol oxidase, but linked with the supply of the H-donor which reduces the quinone.

6. The H-donor for the quinone appears to be the amino-acid group.

Part of the apparatus used in this work was purchased with the aid of a grant by the Government Grant Committee of the Royal Society. I wish to express my gratitude to Professor R. D. Haworth of this University for a number of the phenols used in this investigation and for the opportunities I have had of discussing with him the problems of phenol oxidation.

LITERATURE CITED

- ADAMS, M. H., and NELSON, J. M., 1938: On the Nature of the Enzyme Tyrosinase. *Journ. Amer. Chem. Soc.*, lx. 2472.
- BAKER, D., and NELSON, J. M., 1943: Tyrosinase and Plant Respiration. *Journ. Gen. Physiol.*, xxvi. 269.
- BALL, E. G., and CHEN, T.-T., 1933: Studies on Oxidation-Reduction. XX. Epinephrine and Related Compounds. *Journ. Biol. Chem.*, cii. 691.
- BANGA, I., and SZENT-GYÖRGYI, A., 1938: Dioxymaleinsäure-Oxydase. *Z. Physiol. Chem.*, cciv. 57.
- BOSWELL, J. G., and WHITING, G. C., 1938: A study of the Polyphenol Oxidase System in Potato Tubers. *Ann. Bot.*, N.S., ii. 847.
- GRAUBARD, M., and NELSON, J. M., 1935: Tyrosinase Action on Mono- and Dihydric Substrates. *Journ. Biol. Chem.*, cxi. 757.
- GREGG, D. C., and NELSON, J. M., 1940: A Comparison of the Activities of Tyrosinase toward Phenol and p-Cresol. *Journ. Amer. Chem. Soc.*, lxii. 2506.
- HAPPOLD, F. C., and RAPER, H. S., 1925: The Tyrosinase-Tyrosine Reaction. *Biochem. Journ.*, xix. 92.

- HUBBARD, S. S., 1938: Reversible Action of Oxidised Phenols in the Deamination of certain Amino-acids. *Journ. Biol. Chem.*, cxxvi. 489.
- JACKSON, H., 1939: The Oxidation of Catechol and 1:2:4 Trihydroxybenzene by Polyphenol Oxidase. *Biochem. Journ.*, xxxiii. 1452.
- JOHNSON, S. W., and ZILVA, S. S., 1937: The Oxidation of l-Ascorbic Acid by Plant Enzymes. *Biochem. Journ.*, xxxi. 438.
- KEILIN, D., and MANN, T., 1938: Polyphenol Oxidase, Purification, Nature and Properties. *Proc. Roy. Soc. B*, cxxv. 187.
- LUDWIG, B. J., and NELSON, J. M., 1939: Inactivation of Tyrosinase in the Oxidation of Catechol. *Journ. Amer. Chem. Soc.*, lxi. 2601.
- NEUBERG, C., and SCHWENK, E., 1915: Die Gärung der Dioxymaleinsäure. *Biochem. Z.*, lxxi. 104.
- ONSLow, M. W., and ROBINSON, E., 1928: Oxidising Enzymes. X. The Relationship of Oxygenase to Tyrosinase. *Biochem. Journ.*, xxii. 1327.
- PUGH, C. E. M., 1930: The Mode of Action of Tyrosinase. *Biochem. Journ.*, xxiv. 1442.
- , and RAPER, H. S., 1927: The Action of Tyrosinase on Phenols. *Biochem. Journ.*, xxi. 1370.
- RICHTER, D., 1934: The Action of Inhibitors on the Catechol Oxidase of Potatoes. *Biochem. Journ.*, xxviii. 901.
- ROBINSON, M. E., and MCCANCE, R. A., 1925: Oxidative Deamination by a Basidiomycete Enzyme. *Biochem. Journ.*, xix. 251.
- STEWART, F. C., and PRESTON, C., 1940: Metabolic Processes of Potato Discs under Conditions Conducive to Salt Accumulation. *Plant Physiol.*, xv. 23.
- , STOUT, P. R., and PRESTON, C., 1940: The Balance Sheet of Metabolite for Potato Discs showing the Effect of Salts and Dissolved Oxygen on Metabolism at 23° C. *Plant Physiol.*, xv. 409.
- STONE, R. W., 1937: Ascorbic Acid Oxidase and the State of Ascorbic Acid in Vegetable Tissues. *Biochem. Journ.*, xxxi. 508.
- SWEDIN, B., and THEORELL, H., 1940: Dioximaleic Acid Oxidase Action of Peroxidase. *Nature*, cxlv. 71.
- SZENT-GYÖRGYI, A., 1938: Über die dehydrierende Autoxydation und die biologischen Oxydationen. *Z. Physiol. Chem.*, ccliv. 147.
- WAGREICH, H., and NELSON, J. M., 1936: On the Oxidation Product of Catechol when Oxidised by Means of Tyrosinase. *Journ. Biol. Chem.*, cxv. 459.
- , 1938: On the Mechanism of the Catechol-Tyrosinase Reaction. *Journ. Amer. Chem. Soc.*, lx. 15451.

Studies in Tropical Fruits

XVI. The Distribution of Tannins within the Banana and the Changes in their Condition and Amount during Ripening

BY

H. R. BARNELL

AND

E. BARNELL

*(Low Temperature Research Station, Imperial College of Tropical Agriculture,
Trinidad, B.W.I.)*

With nine Figures in the Text]

I. PREFACE

THE banana fruit when green and in the early stages of ripening is astringent and certain cells of both pulp and skin have contents which give positive tests with tannin-staining reagents. The pulp of the well-ripened fruit is non-astringent and notable changes are observed to have occurred in the contents of the tannin-containing cells.

The present paper deals with the tannins and their changes in the Gros Michel banana fruit. Its scope is twofold: in the first part the distribution of the tannins within the tissues and the changes observed in them have been followed microscopically; in the second part a description is given of the development of a technique for the estimation of 'active' (astringent) tannins and its use in following quantitatively the changes in the active tannins in pulp and skin during ripening.

II. DISTRIBUTION OF TANNINS IN THE TISSUES OF THE BANANA FRUIT

(a) Introduction

The few available publications on anatomical investigations of tannin distribution in the banana fruit have been chiefly concerned with the effects of 'chilling' (overlong exposure to low temperature during storage). Even in this restricted field there is need of a basis for comparison consisting of detailed observations on tannin changes through a series of developmental stages in the normally ripening fruit.

Scurti and Pavarino (1933), working on the effects of low temperatures on stored bananas, described only 'secretion canals' of normally ripening mature fruits as being rich in tannins. They associated the initiation of ripening with the coagulation of the latex in these canals and with the disappearance of soluble tannins. Slocum (1933) also observed this tendency of the latex to

coagulate as the fruit ripens. Wardlaw and McGuire (1931) made brief reference to tannins contained in 'isolated cells' and in 'mucilage ducts'.

In the present work the anatomy of the 'three-quarter full' Gros Michel banana fruit has been examined from the freshly cut green to the eating-ripe condition during ripening at tropical temperatures. Attention has been paid to identifying tannin-containing cells and noting the visible changes occurring in their contents.

(b) Methods

The staining reagents used were potassium dichromate and ferric chloride, both in 5 per cent. solution. The observations made therefore refer only to tannins which give the characteristic colour reactions with these reagents. Sections of green 'fingers'¹ were mounted in glue² to prevent the flow of latex from latex vessels into adjoining cut cells. Sections of the pulp of unripe fruit were treated with concentrated hydrochloric acid to remove the starch grains which otherwise completely obscured the tissue structure.

Drawings were made with the aid of a camera lucida, the magnification used for detailed examination of the tissue structure being 144.

(c) Anatomy of the fruit

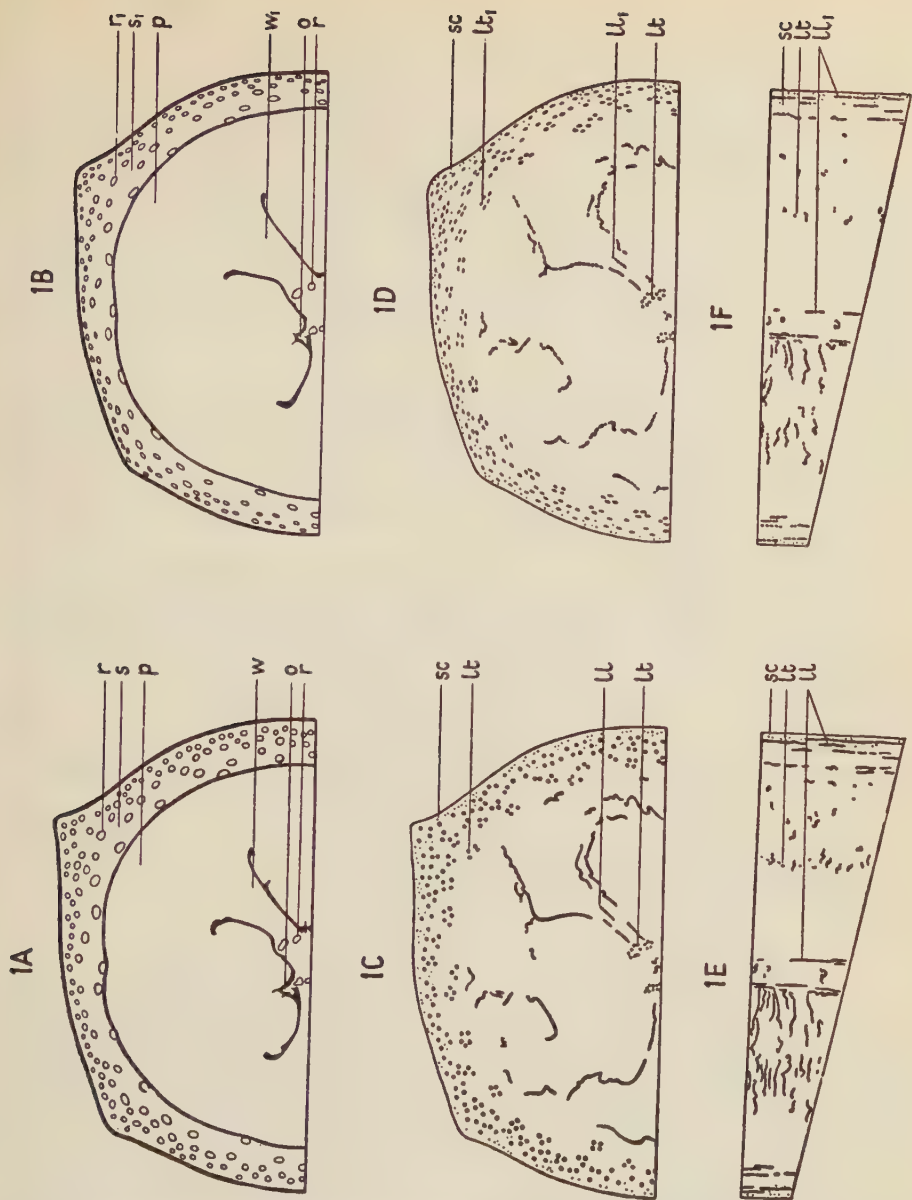
Examination by eye and with the aid of a lens of a green finger cut transversely across the centre (Fig. 1) shows the following regions: (1) the skin which encircles the pulp and has a thickness approximately one-fifth the diameter of the pulp. It has a sharply ridged outer margin and its boundary with the pulp is clearly marked by the colour contrast with the white, starchy pulp tissue, (2) the pulp with its sterile ovules contained within compressed locules and the three arms of its inner locular walls extending radially from the centre.

The positions of the vascular strands are easily visible to the naked eye. They are scattered abundantly in the skin and grouped in three pairs in the centre of the pulp with numerous branches ramifying through the rest of the pulp. The strands are clearly marked by the nature of adjoining cells, viz. the latex vessels associated with all the bundles, the pericycle fibres of outer skin bundles, and the large parenchymatous cells surrounding the middle and inner skin bundles.

During ripening considerable changes are observed. The skin becomes yellow and less succulent, decreasing in thickness and showing tangential stretching in the lessened sharpness of its ridges and the distortion of the tissues, particularly the aerenchymatous tissue and the region of the vascular bundles with their associated latex vessels. The pulp assumes a yellow satin-like appearance, while the three radial locular walls increase slightly in thickness.

¹ For explanation of terms see Wardlaw, Leonard, and Barnell (1939).

² Hoof glue, locally known as 'brown glue', dissolved in water with alcohol added in amount sufficient nearly to precipitate the glue.



FIGS. 1A-F. Half transverse sections of $\frac{3}{4}$ -full Gros Michel banana: 1A, green; 1B, eating-ripe, illustrating major anatomical features. ($\times 4$.) *r*, vascular tissue and latex vessel region; *r*₁, region of skin showing tangential stretching; *s*, *s*₁, skin showing decrease in thickness; *p*, pulp; *w*, inner locular wall; *w*₁, swollen inner locular wall; *o*, ovule in loculus much compressed by ingrowth of outer locular wall. 1C, 1D, the same fruits showing distribution of tannin-containing elements. *lt*, latex vessels cut transversely; *lt*₁, latex vessels tangentially stretched; *sc*, small scattered tannin-containing cells; *ll*, latex vessels in longitudinal view; *ll*₁, latex vessels with tannin contents reduced and withdrawn from walls. 1E, 1F, radial longitudinal sections of same fruits showing distribution of tannin-containing elements. *lt*, latex vessels cut transversely; *sc*, small scattered tannin-containing cells; *ll*, latex vessels in longitudinal view; *ll*₁, latex vessels in longitudinal section with tannin contents reduced and withdrawn from walls.

(d) Distribution of tannin-containing cells

The most conspicuous of the tannin-containing units are the numerous latex vessels which extend longitudinally through the skin and through the central part of the pulp; from the centre they branch more or less radially across the bulk of the pulp (Figs. 1–3). Each vessel is composed of a number of approximately cylindrical cells (Figs. 2, 4, and 5) constricted at their ends so that, although cross walls are absent, the diameter of the circular opening between cells is very much smaller than the diameter of any other part of the cell.

In unripe, green bananas the viscid and liquid latex is under pressure and flows freely when the finger is cut. In 'sprung'¹ fruit the flow is greatly diminished,² the latex collecting in small beads on the cut surface. In ripe, edible fruit the latex has apparently lost water and changed to such an extent that it is 'caked' and brittle, lying in the centre of the cell withdrawn from the walls (Figs. 6 and 7).

The latex vessels are closely associated with vascular bundles (Fig. 2) and vary somewhat in size. They occur singly or in pairs near small bundles in the outer and middle skin (Fig. 3) and in the mass of the pulp (Fig. 4); they form rings around the larger bundles of the inner skin (Fig. 3) and centre pulp (Fig. 5).

Tannins are detected also in small scattered isolated cells with dense contents occurring in the outer and middle skin not associated with vascular bundles.

As the fruit ripens tannin colour reactions are observed in cells hitherto free from tannin contents. These are mainly to be found near latex vessels or associated with scars and markings on the skin.

(e) Changes in the cell tannin contents during ripening

In the three-quarter full, green fruit tannins are confined to the latex vessels of the pulp and skin and to the small scattered cells of the outer and middle regions of the skin.³ Except for small oil globules⁴ in the latex vessels of the skin, the contents of these cells and vessels stain evenly with potassium dichromate and ferric chloride, indicating a uniform distribution of tannins in each cell.

After the finger has sprung the latex in the pulp tends to 'cake'. Simultaneously there is an apparent increase in the amount of oil present in the latex vessels of the skin. At no stage is oil observed in the vessels of the pulp.

As ripening progresses the latex of the skin undergoes changes similar to

¹ For explanation of this term see Barnell (1941).

² The latex flow appears to increase slightly just before the finger attains the sprung condition. Confirmation of this by measurement has not yet been attempted.

³ An occasional vessel or fibre contains tannin. This may result from the incomplete 'fixing' of latex exuding from the vessels after treatment with glue.

⁴ Slocum (1933) refers to these as 'spherical particles of solid matter in a state of suspension' giving a cloudy appearance to the latex of green fruit. Scurti and Pavarino (1933) also note their presence.

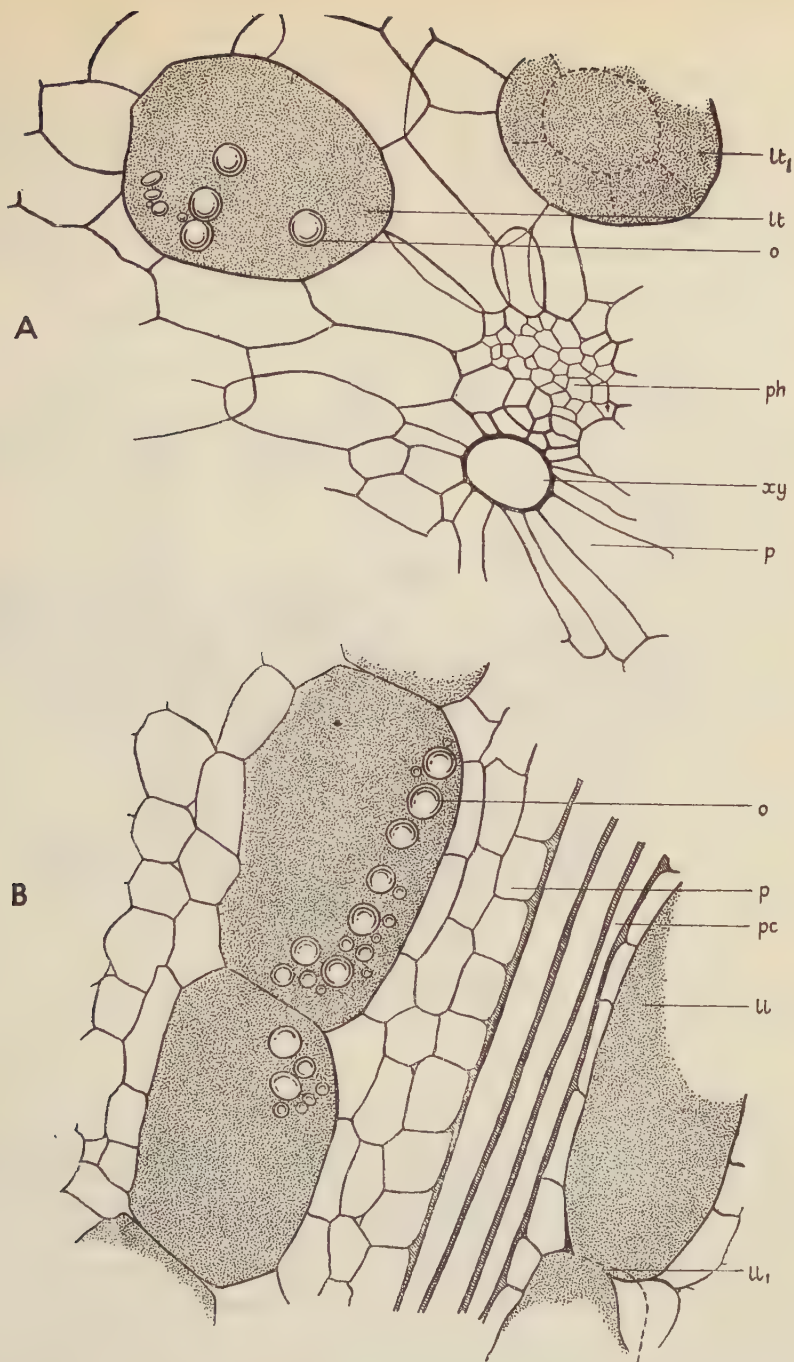


FIG. 2. Skin of 3/4-full green banana. ($\times 144$.) A, Transverse section: *lt*, latex vessel cut through centre, shading indicates liquid tannin-containing contents; *lt*₁, latex vessel cut near junction of cells; circular dotted lines indicate constricted opening between cells at slightly lower plane; *o*, oil globule; *p*, parenchymatous cells; *ph*, phloem; *xy*, xylem vessel. B, Longitudinal section: *ll*, latex vessel; *ll*₁, latex vessel cut through centre showing constricted opening between cells; dotted line indicates apparent cross wall when cut is not through the centre of the cell; *o*, oil globule; *p*, parenchymatous cells; *pc*, pericycle fibre.

those in the pulp (Fig. 6), all the latex of the fruit eventually becoming caked and brittle and withdrawn from the walls giving a beaded appearance to each chain of cells¹ (Fig. 7). In the pulp first and subsequently in the skin, tannins gradually disappear from these dried latex contents. The former rich brown stain given with potassium dichromate is replaced by a pale pink; eventually

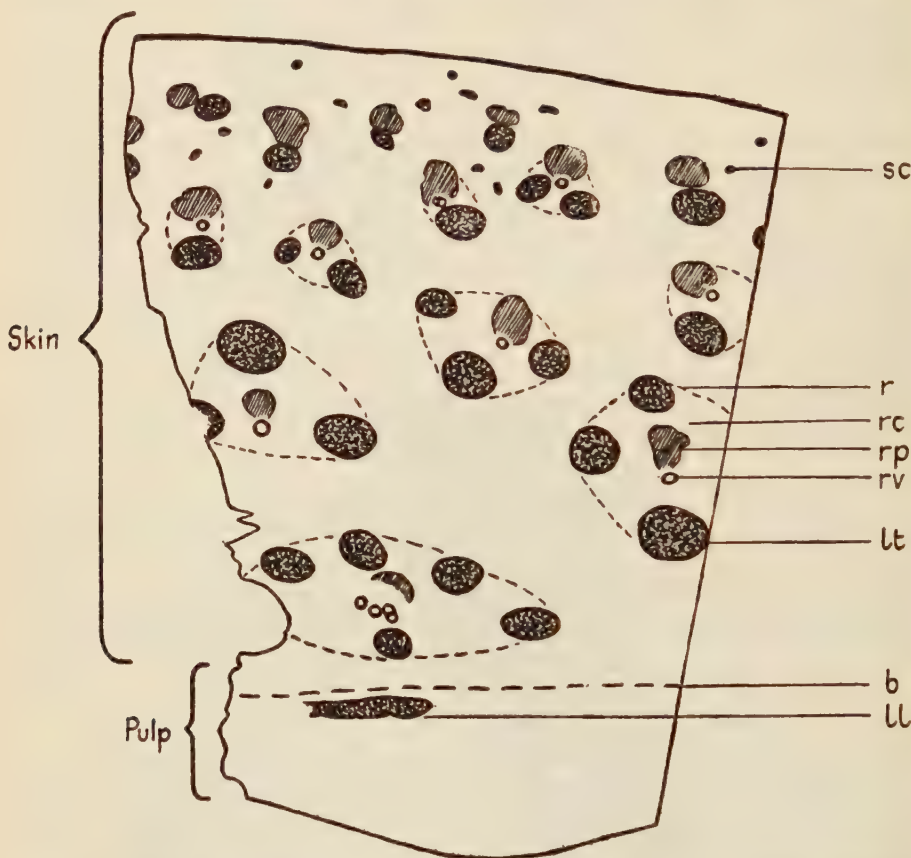


FIG. 3. Transverse section of skin of $\frac{1}{4}$ -full green banana: Distribution of tannin-containing elements. ($\times 32$.) *b*, line demarking skin and pulp; *sc*, small scattered tannin-containing cells; *r*, line delimiting pericycle region (*rp*), vascular tissue (*rv*), large parenchymatous cells (*rc*), and latex vessels cut transversely (*lt*); *ll*, latex vessel of pulp cut longitudinally.

no coloration is observed. All these stages may be observed in the pulp, and to a less extent in the skin, of the yellow-green eating-ripe fruit. The contents of the small scattered cells of the skin continue to stain darkly. During ripening tannins appear in the cells in close proximity to latex vessels. These may be parenchymatous cells, xylem vessels, or fibres. In the pulp this development of tannins is limited and confined to the region of the six central vascular strands, but in the skin it occurs fairly extensively in cells

¹ Exceptions to this occur infrequently. The latex sometimes adheres to the walls leaving a central vacuole.

near latex vessels or in the outer tissues where injury has occurred and markings have appeared on the surface.

As the finger ripens the oil globules in the latex vessels of the skin tend to coalesce¹ within the caked contents.

III. THE DIASTASE METHOD OF ESTIMATING 'ACTIVE' TANNINS, AND ITS USE IN FOLLOWING CHANGES IN ASTRINGENCY IN THE BANANA

(a) Introduction

A feature of the unripe banana is the strongly astringent taste of both pulp and skin which does not entirely disappear in the ripened fruit and which



FIG. 4. Pulp of $\frac{3}{4}$ -full green banana. Longitudinal section through inner locular wall. ($\times 144$.) ll, tannin-containing latex vessel; xy, xylem vessel; pc, pericycle; p, parenchyma; i, intercellular space.

remains particularly noticeable in fruits of inferior quality such as those which have been chilled (Barnell, 1943) or gassed (Wardlaw, 1940) or those from plants infected with *Cercospora* leaf-spot disease (Barnell, 1940). Unripe bananas contain a considerable amount of easily demonstrable tannins (section I) which do not completely disappear on ripening. Investigations concerned with quality in banana fruit must therefore take account of the tannins present, and some method of assessing their amount, or more strictly the amount of astringent tannic substance, is required. The present work describes an investigation into this aspect of the eating quality of the banana.

Degrees of astringency as determined organoleptically and amounts of tannin as estimated by the usual methods cannot be closely correlated. As far back as 1789 Cullen, in criticizing Bergius's (1778) method of estimating

¹ Slocum (1933) observes this coalescence of the globules upon ripening into "gummy" amorphous masses which leaves the latex of ripe bananas clear.

astringency by comparing the colours produced with iron salts, held that there is no direct relationship between the amount of tannin present and the astringency of the material. This view has been supported by the work of Phillips (1922) and Crede (1925). It is possible, then, that only part of the tannin present in any material is responsible for producing astringency, and it is noteworthy that the astringency appears to coincide with the tanning activity of a tan liquor. Thus Wilson (1928) states that 'when a tan liquor is tasted, this produces on the tongue the sensation of puckering. Materials causing this effect are called astringent in proportion to the vigor of the effect. Astringent tanning materials tan rapidly and so the rate of tanning in a given tan liquor has sometimes been used as a measure of astringency of the liquor.'

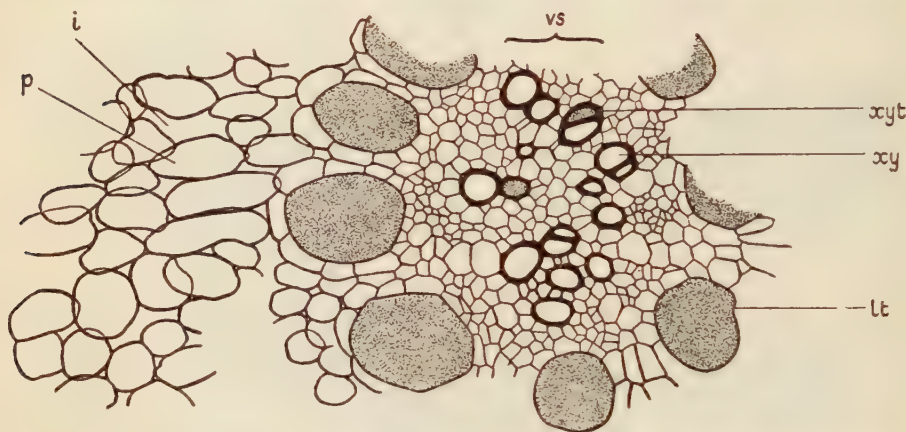


FIG. 5. Pulp of $\frac{3}{4}$ -full green banana. Transverse section of vascular bundle in centre of pulp. ($\times 144$.) *vs*, one of six main vascular strands supplying pulp; *xy*, xylem vessel; *xyt*, xylem vessel with tannin contents; *lt*, one of the surrounding ring of tannin-containing latex vessels; *p*, parenchyma; *i*, intercellular space.

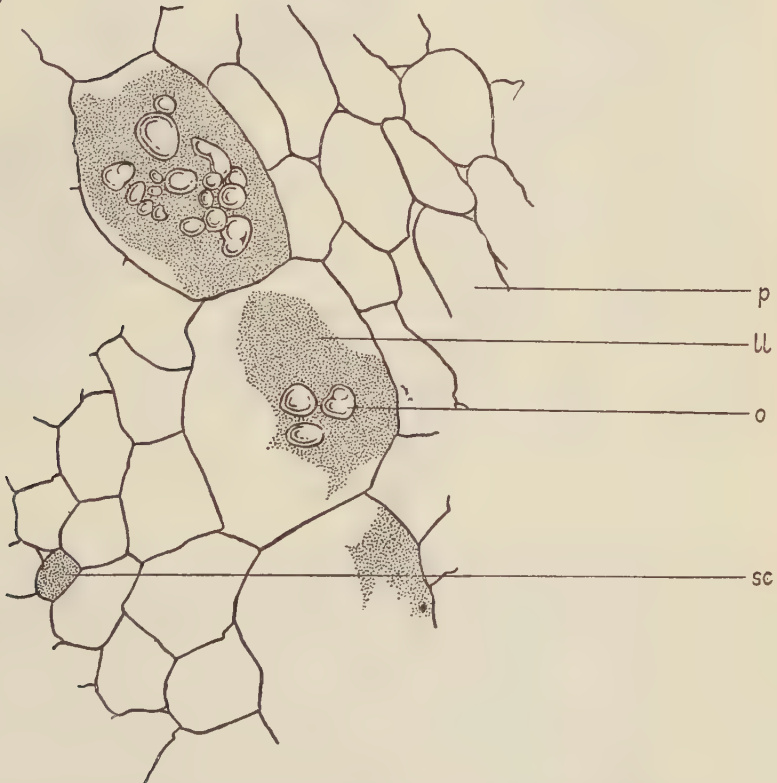
Classical methods of estimating tannins such as the Lowenthal (Allen, 1927), cinchonine sulphate or quinine sulphate precipitation (Chapman, 1907; Tatlock and Thompson, 1910), which give the total amounts of tannin present, may be of little value in assessing the importance of tannin in the quality of fruit. For example, a set of values is available for the relative amounts of tannin in the pulps of unripe and ripe bananas of varieties grown in Porto Rico (Toro, 1922), in which an increase in total tannin in the pulp on ripening is shown for each of the varieties examined.

In the persimmon the tannin present in the unripe fruit does not disappear but enters into a closer combination with a specific colloid of carbohydrate nature, an insoluble gel eventually being produced (Lloyd, 1911, 1912). In this form tannin is no longer astringent. Similar conclusions as to the relations of tannins with colloids in fruit have been drawn by Michel-Durand (1928, 1929, 1930) and Hubert (1924) from the amounts of tannin extracted with various water-acetone solutions.

6A



6B



FIGS. 6A & B. Skin of $\frac{3}{4}$ -full eating-ripe banana. ($\times 144$.) A, Transverse section. B, Longitudinal section. *lt*, *ll*, latex vessels with caked tannin-containing contents withdrawn from walls; *o*, oil globules coalescing; *p*, parenchyma; *xy*, xylem vessel; *pc*, pericycle fibres; *sc*, small tannin-containing cell.

(b) Methods and materials

Brown and Morris (1893) observed that diastase is precipitated or otherwise inactivated in the presence of tannin. This observation has been utilized in the present attempt to estimate the amounts of active tannins in the banana. The amount of inhibiting substance is estimated in this method

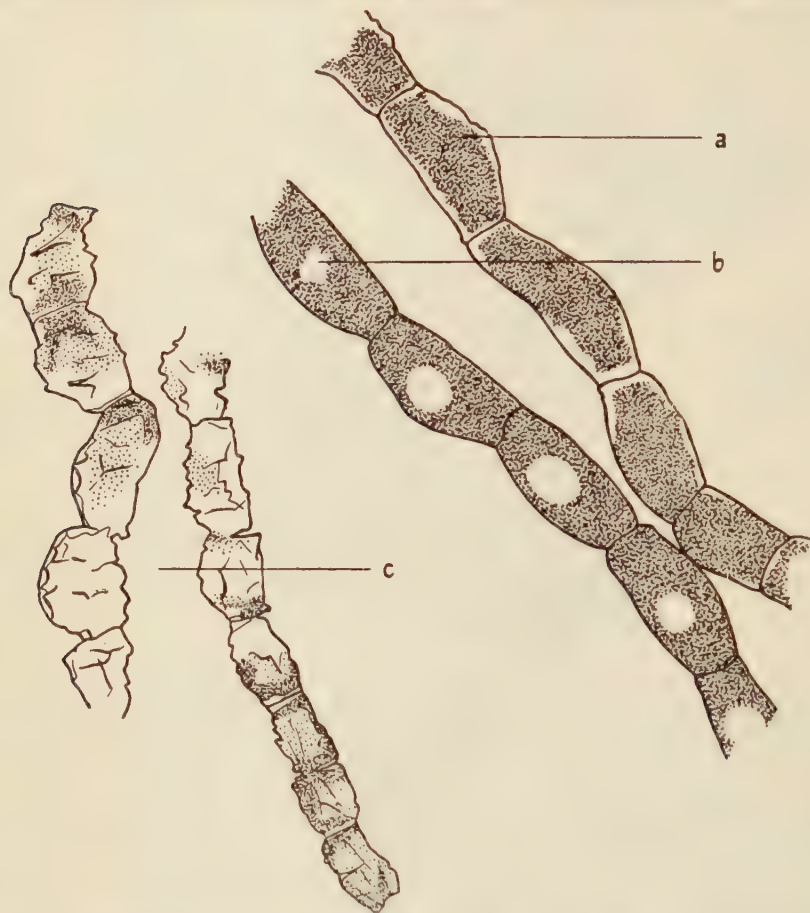


FIG. 7. Changes in contents of latex vessels upon ripening. ($\times 48$.) Stages in disappearance of tannins: *a*, tannin-bearing contents withdrawn from walls; *b*, contents withdrawn from cell centres leaving vacuole (exceptional); *c*, cells with contents caked and cracking.

from the difference between the time required for the hydrolysis of a given amount of soluble starch by a standard diastase preparation under standard conditions (*a*) in the presence of an aqueous solution of the substance, (*b*) in the presence of water.

It was found necessary to vary the composition of the digest according to the concentration of the inhibiting material. For relatively high concentrations the method which will be referred to as 'standard' required the following reagents: (1) 25 ml. 2 per cent. filtered 'Analar' soluble starch; (2) 20 ml.

filtered tannin-containing solution or water; (3) 2 ml. acetate buffer, pH 4·7; (4) 2 ml. freshly prepared 0·0125 per cent. taka-diastrase solution.

The starch solution is pipetted into a large boiling-tube (200×80 mm.), this is followed by the test solution or the water, the buffer solution, and, finally, the standard taka-diastrase solution (a filtered solution of Parke Davis's undiluted preparation). As the diastrase is added a stop-watch is started, the tube shaken, and a dropping tube inserted. Samples are withdrawn at one-minute intervals, and two drops added to one drop iodine solution (5·2 gm. iodine, 7·6 gm. potassium iodide per litre) on a glazed plate. The end-point adopted was the first appearance of a clear violet colour, or absence of the characteristic starch-iodine blue. The appearance of this colour marks the hydrolysis of a definite amount of starch each time, with the concomitant appearance of dextrans. The colour is easily observed and, after some practice, very consistent results may be obtained. All observations have been made at tropical temperatures, 28–31° C., the use of a blank in every determination obviated the necessity for a closely controlled temperature.

The amount of active tannin or diastrase-inhibitor present was found over the range of concentrations encountered in the banana to be proportional to $\log t - \log t_0$, where t is the time in minutes for the starch hydrolysis in the presence of the inhibitor and t_0 is the time taken in the blank. For low concentrations of inhibitor the method was modified by increasing the volume of starch and decreasing the quantity of taka-diastrase as follows: 50 ml. 2 per cent. filtered 'Analar' soluble starch, 20 ml. test solution or water, 2 ml. acetate buffer, pH 4·7, 1 ml. 0·0125 per cent. taka-diastrase.

Table I sets out the data obtained for two dilution experiments carried out with freshly prepared solutions of tannic acid.¹ It was necessary to prepare the solutions immediately before use as their inhibitory activity towards diastrase decreases on standing. The data are plotted in Fig. 8, and it will be observed that the numerical results lie closely along a straight line. Similar straight-line relationships have also been obtained in dilution experiments

TABLE I
Dilution Experiments with Tannic Acid

	Tannic acid (mg. per 200 ml. test solution).	Standard units per 20 ml.	
		A	B
Modified method	25	0·093	0·126
	50	0·156	0·174
	80	0·306	0·335
Standard method	50	0·176	0·176
	100	0·398	0·368
	150	0·580	0·556
	200	0·763	0·748

Variance of an observation = 6·3%. Sig. diff. between observations = 0·028
($P = 0·05$)

¹ The tannic acid was obtained from the Chemistry Department of the Imperial College of Tropical Agriculture and had long been in stock.

TABLE II
Removal of Inhibitor by Hide-powder

	Water blank.	Skin extract after hide-powder treatment.	Water after hide-powder treatment.	Skin extract.	Water plus taka-diestase after hide-powder treatment.
Time (min.) to starch disappearance	6	6	6	37	6

TABLE III
Removal of Tannin-Diastase Complex by Hidepowder

	Water blank.	Skin extract.	Skin extract after hide-powder treatment.	Skin extract plus taka-diastase after hide-powder treatment.
Time (min.) to starch disappearance	6	22	6	23

with aqueous extracts of banana pulps and skins. For the tannic acid data the variance of a single observation was 6.3 per cent., whilst the significant difference ($P = 0.05$), from Fisher's table of t , was 0.028. Accordingly, a reasonably high level of accuracy may be claimed for this method.

The method has not been calibrated, since, due to their instability, the tannins of the banana have not yet been isolated in sufficient purity. All results are expressed in 'standard units' which assess the inhibitory activity of the solutions tested, and, by assumption, their astringency. A factor of 0.781 was used to convert values obtained by the 'modified' method used for dilute concentrations to 'standard' units. This factor is the mean of twenty-four values, each value being the ratio of an observation obtained by inspection from the graph in Fig. 8 to a corresponding observation obtained using the modified method.

Removal of inhibitor by hide-powder. Evidence that the inhibitor of diastase activity was a tannic substance was obtained by passing an aqueous extract of banana skin through a tube filled with hide-powder. Fifty millilitres each of banana-skin extract, of water+taka-diastase, and of water alone were filtered through tubes of hide-powder and then, together with the same volume of the untreated skin extract, an estimation of the inhibitor content carried out. The values obtained for the starch-disappearance time are given in Table II; they show (i) that the inhibitor was completely removed by the hide-powder (columns 2 and 3) and (ii) that the hide-powder treatment had no effect on the activity of the taka-diastase (column 6), since water receiving the same treatment (column 4) gave the same blank value.

Data have also been obtained showing that, while taka-diastase can pass through hide-powder without loss of activity, the tannin-diastase complex is quantitatively removed. Table III gives the results for a banana-skin extract when (a) untreated (column 3), (b) receiving hide-powder treatment (column 4),

(c) receiving hide-powder treatment after addition of standard amount of taka-diascase (column 5). The hide-powder completely removed the inhibiting activity of the skin extract, but, when the taka-diascase was added before passing the extract through hide-powder, the taka-diascase was proportionately removed, with the result that the starch-disappearance time was approximately equal to that in the presence of the untreated skin extract.

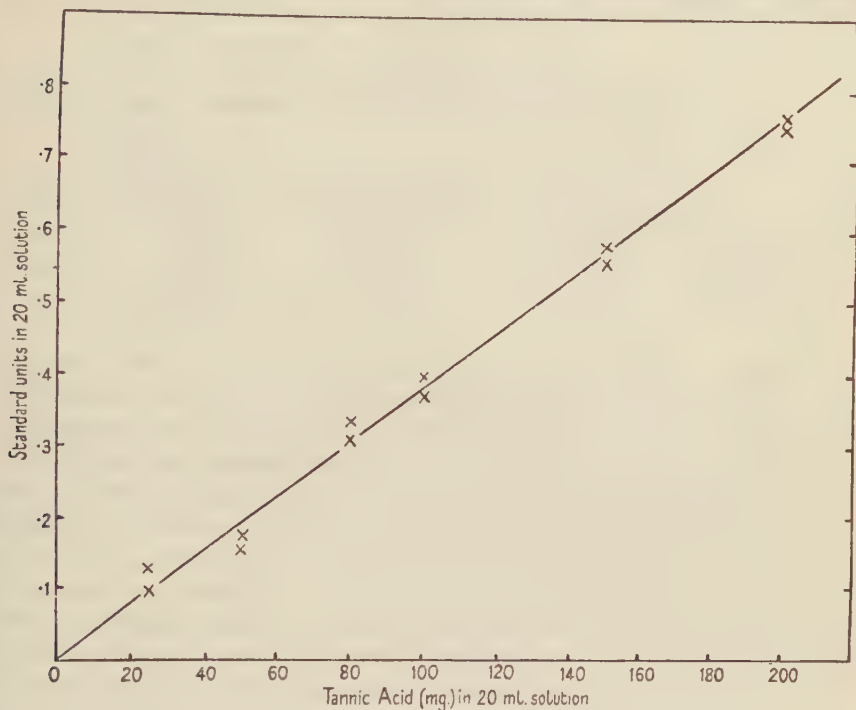


FIG. 8. Relation between tannic acid concentration and value, in arbitrary units, obtained by the method for the estimation of 'active' tannin. Data from Table I.

This shows that the tannin-diascase complex as well as free tannin is removed by hide-powder.

Effects of standing and of sugar concentrations on the estimation. (a) An aqueous extract of banana pulp or skin darkens to a brownish hue on standing. This has been observed to be accompanied by a decrease in the inhibitory activity of the extract towards taka-diascase (Table IV, columns 3 and 4). (b) Aqueous extracts of the pulp and skin of the green banana contain very low sugar concentrations, while those from the pulp of the ripe banana in particular have high concentrations. It was necessary, therefore, to determine the effect, if any, of sugar concentrations on this method of estimating tannic substances. The values shown in columns 5 and 6 of Table IV demonstrate that a moderate concentration of invert sugar exerts no effect.

Effect of banana diastase. During extraction any banana diastase liberated into solution presumably combines with the tannin in solution to form a

TABLE IV

Effects of (a) Standing and (b) High Sugar Concentrations

	Water blank.	Pulp extract (a).	Pulp extract (a) after standing for 5 days.	Pulp extract (b).	Pulp extract (b) in 4.5% invert sugar.
Time (min.) to starch disappearance	6	34	23	23	23

TABLE V

Extraction of 'active' Tannin by Toluene-saturated Water

	1st extraction (units/100 gm.).	2nd extraction (units/100 gm.).	1st extraction as percentage of total extraction.
A pulp	7.43	1.06	87.5
B pulp	7.31	1.51	82.9
A skin	38.2	4.00	90.5
B skin	42.8	3.70	92.0

tannin-diestase complex; other proteins present may also combine. Although it has been shown that aqueous solutions of diastase will pass through hide-powder without loss of activity, and that the inhibitor is quantitatively removed, it has also been shown that the tannin-diestase complex is quantitatively removed. The free tannin estimated in any aqueous extract of banana tissue must be the excess present after the deactivation of some by liberated banana diastase and possibly other proteinaceous substances. Part of the decrease in amount of tannin shown below (section (c)) to occur in both pulp and skin may be attributed to increasing amounts of diastase present in pulp and skin.

Extraction time. Extraction was carried out with toluene-saturated water at room temperature (29° C., approximately), the active tannin substances in the banana being very soluble in water as shown by the following tests:

Table V presents data for the amounts extracted from the pulps and skins respectively of two bananas (a) by extraction overnight, (b) by a further 24-hour extraction of the same tissues with fresh water. In each instance the tissue was placed in an air-tight glass vessel with 100 ml. of water saturated with toluene. Over 80 per cent. of the total amount extractable in the two extractions was obtained from the pulps in the first 12-hour period; in the skins the amount was over 90 per cent. It is reasonable to consider, then, that an overnight extraction of freshly sliced tissues with water at room temperature will remove 80–90 per cent. of the free tannin substance; this extraction time was used throughout the present work.

Materials. Bunches as free as possible under local conditions from *Cercospora* leaf-spot disease (Wardlaw, 1937) were selected for the investigations on changes of tannin contents during ripening. For the three experiments at tropical temperatures six Gros Michel 'heavy $\frac{3}{4}$ -full' bunches were obtained and from these two healthy ones selected by observation of the

colour of freshly broken fingers, those with fingers showing any trace of the pinkish-buff coloration characteristic of *Cercospora* infection were rejected. The bunches were stored at a relatively steady temperature in one of the storage rooms.

Single fingers were removed at suitable intervals of time from the upper rows of the third hands of the two bunches for extraction and estimation. Skins were separated from pulps; the latter were finely sliced and the former cut with scissors into small pieces for extraction.

For the experiments on changes during storage at 53° F. and 68° F. fifteen healthy 'heavy $\frac{3}{4}$ -full' bunches were selected. Five similar bunches were available for the investigation of bananas maintained continuously at 53° F. Bunches were placed in store at 53° F. within 24 hours of cutting. Sampling consisted of removing one finger from the upper row of the third hand of each bunch in the population, separating the fingers into skin and pulp, freezing at approximately -10° F., powdering and sub-sampling the frozen powder. The frozen material could be retained at 0° F. for several days without appreciable loss of active tannin.

(c) Experimental data

Changes in the active tannin content of pulp and skin during the ripening of the banana at tropical temperatures. The data for the changes in active tannin content of the pulp and skin of the banana during ripening at tropical temperatures are set out in Table VI. Each value is the mean of two

TABLE VI

Changes in the Amount of 'active' Tannin in Pulp and Skin of 'heavy $\frac{3}{4}$ -full' Bananas during Ripening at Tropical Temperatures (expressed as standard units per 100 gm. of tissue)

Days.	Series 1			Series 2			Series 3		
	Fruit condition.	Pulp.	Skin.	Fruit condition.	Pulp.	Skin.	Fruit condition.	Pulp.	Skin.
0	Green	7.36	40.5	Green	—	—	Green	—	—
1	"	8.01	34.0	"	7.47	30.9	"	—	—
2	"	7.57	28.3	"	6.75	27.2	"	—	—
3	"	4.30	25.4	"	7.02	18.1	"	11.1	30.2
4	"	5.02	25.9	"	8.51	17.9	"	—	—
5	Colouring	4.30	16.5	Colouring	3.50	23.9	Colouring	12.0	30.3
6	"	3.87	18.1	Eating-ripe	4.35	11.9	"	—	—
7	"	1.95	11.2	"	2.57	4.83	Eating-ripe	3.02	4.01
8	Eating-ripe	2.84	4.58	Over-ripe	1.43	2.20	"	—	—
9	"	1.99	4.72	"	1.13	1.89	"	1.52	2.02
10	Over-ripe	2.00	4.51	"	—	—	Over-ripe	1.26	2.17
11	"	1.32	3.51	"	1.07	2.25	"	1.37	2.52
12	—	—	—	—	—	—	"	1.35	2.17
13	—	—	—	—	—	—	"	1.38	2.97
Sig. diff. (<i>P</i> = 0.05)	—	1.68	6.17	—	1.66	3.68	—	2.92	8.52

All estimations shown in the columns headed Series 1 and Series 2 were made with the 'standard' method, which is not sensitive enough for the small quantities noted in pulps and skins of ripe and over-ripe bananas. In Series 3 the values from the 9th day onwards were obtained by the 'modified' method. In subsequent tables all estimations of ripe and over-ripe fruit were made with the 'modified' method.

Sig. diffs. for last five values of Series 3 were: 0.30, 0.28.

determinations for upper row fingers of the third hands (or, towards the end of each experimental period, the fourth hands) of two selected bunches and is expressed as a percentage in standard units. The stages of ripening of the bunches are indicated for each of the three trials. Minimal significant differences between observations are shown at the foot of each column.

At the time of harvesting the percentage of active tannin in the skin ranged from four to five times that present in the pulp. The percentage in the skin fell, with some variability due to ripening irregularities, from the day of cutting until approximately the appearance of anthracnose spotting on the skins (over-ripe stage) (Wardlaw, Leonard and Barnell, 1939a, p. 16).

In the pulp the data indicate, in general, that after cutting there was a delay before the concentration of active tannin began to fall. The fall began shortly before the fruit attained the sprung condition and began to change colour.

During the eating-ripe and over-ripe stages there was little change in the active tannin content of both pulp and skin; the skin containing during this period approximately twice the percentage of active tannin found in the pulp.

Changes in the active tannin content of pulp and skin of bananas at 53° F. and 68° F. Table VII records the percentage amounts in standard units of

TABLE VII

Changes in the Amount of 'active' Tannin in Pulp and Skin of 'heavy $\frac{3}{4}$ -full' Bananas at 53° F. and 68° F. (Expressed as standard units per 100 gm. tissue)

Days in store.	Temperature.	Pulp.	Skin.	Pulp/skin weight ratio.
0	53°	3.52	25.5	1.61
2	"	3.52	26.9	1.59
4	"	3.52	26.9	1.60
7	"	1.76	24.1	1.65
10	"	1.76	17.0	1.69
11	"	1.25	15.9	1.65
12	68°	—	—	—
13	"	1.25	7.04	1.75
15	"	1.00	6.02	1.64
17	"	0.94	2.40	1.79
20	"	0.89	2.10	2.28
22	"	0.93	2.51	2.54
Sig. diff. ($P = 0.05$)		0.06	0.95	—

active tannins in 'heavy $\frac{3}{4}$ -full' bananas during a refrigerated storage period at 53° F. for 12 days followed by ripening at 68° F. The values for the pulp and skin are given in columns 3 and 4 respectively, with significant differences at the foot of each column. Column 5 gives values of the pulp/skin weight ratio to indicate the progressive stages of ripeness of the fruit during the storage and ripening periods. Under ripening conditions such as these a value of 2.0 for the ratio is given by fruit in the eating-ripe condition (Barnell, 1941a).

The values from Table VII are plotted for pulp and skin in Fig. 9. The

early values for both skin and pulp were lower than those found in the single finger sampling of the bananas ripening at tropical temperatures (Table 6); the trends of the values were similar though on a time basis much extended by the lower temperature. The initial values were maintained for approximately 4 days at 53° F., after which they fell in both skin and pulp.

The bunches were transferred to 68° F. after 12 days at 53° F. and the majority attained the sprung condition by the 14th day, at which time the

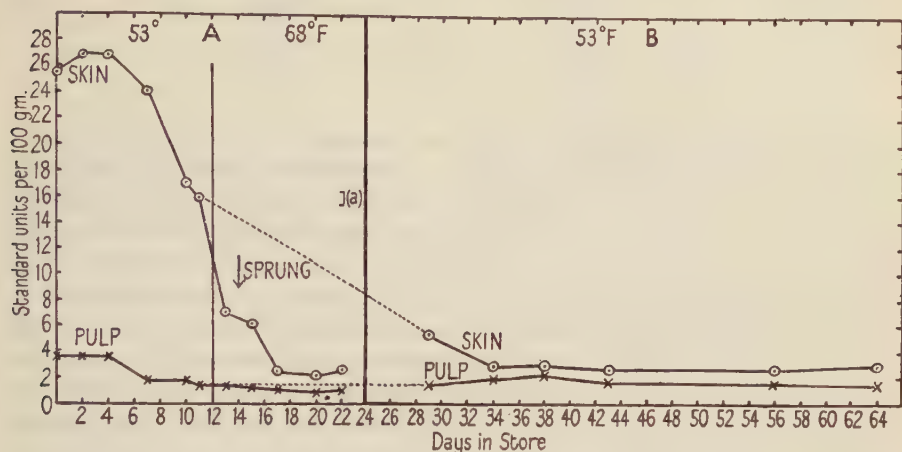


FIG. 9. A, Percentage amounts in standard units of 'active' tannin in pulps and skins of bananas stored at 53° F. and ripened at 68° F. B, Percentage amounts in pulps and skins of bananas stored continuously at 53° F. The two sets of observations on fruit stored at 53° F. are connected by a broken line. The long-stored batch were not sampled until they had been at 53° F. for 29 days. The significant difference ($P = 0.05$) for the skins' values in A is indicated by the vertical line (a); the value for the pulp (0.06) was too small to indicate in this way.

active tannin contents of both skin and pulp had fallen to approximately one-quarter of their respective initial values. In the sprung condition the banana no longer exudes latex when cut or broken (cf. II, section (d)). At 53° F. the onset of rapid starch hydrolysis is considerably delayed (Barnell, 1941a); the effect of this temperature in delaying the onset of rapid decrease in active tannin is much less. The time at which the major carbohydrate changes occur in the ripening banana coincides in general with the beginning of colouring which occurs at approximately the same time as the sprung condition (Wardlaw, Leonard, and Barnell, 1939a, p. 15). It is of interest to note that in the sprung condition the active tannin content of skin and pulp has fallen to relatively low values and continues to decrease to very low values as the banana ripens.

The five bunches maintained continuously at 53° F. were sampled at intervals from the 29th to the 64th day. The data are recorded in Table VIII and plotted in Fig. 9. The active tannin in the skin had, apparently, not reached its lowest value by the 29th day but did so by the 34th; the pulp values were already down to the approximately constant value of 'ripe' fruit

TABLE VIII

Changes in the Amount of 'active' Tannin in Pulp and Skin of 'heavy $\frac{3}{4}$ -full' Bananas during continuous Storage at 53° F. (Expressed as standard units per 100 gm. tissue)

Days in store.	Pulp.	Skin.	Pulp/skin weight ratio.
29	1·32	5·26	2·14
34	1·89	2·87	2·44
38	2·27	2·99	2·86
43	1·65	2·64	2·92
56	1·65	2·64	3·42
64	1·56	3·00	3·54

by the first sampling date (for these 'long storage' bunches). Apart from small variations there was little change in the amounts in either skin or pulp over the whole period, but comparison of the data with those of the bananas ripening at 68° F. shows that the final values were higher in both skin and pulp. The final concentration of active tannin in the pulp was nearly twice that in the pulp of bananas ripened at 68° F. or at tropical temperatures.

Tannin contents of pulps of bananas of various eating qualities. The tannin content of a particular batch of bananas under a particular set of storage conditions showed relatively little change during the eating- and over-ripe stages. It was therefore possible to compare the tannin contents of pulps of bananas of various eating qualities by comparing sequences of determinations spread over these stages.

The material used was (a) healthy fruit ripened under tropical conditions having fairly good eating quality, not equal, however, to that ripening under controlled conditions at a lower temperature, e.g. 68° F. (cf. Barnell, 1941a, p. 641); (b) fruit from plants severely infected with *Cercospora* leaf-spot, the pulp having a pinkish colour, especially while unripe, and a slightly astringent flavour when ripe; (c) fruit which had been severely chilled by long exposure to 53° F. In each instance upper row fingers from the third hand of a bunch were used for the tannin estimation.

Values are set out in Table IX for successive determinations made during the eating-ripe and over-ripe stages of the different fruits. Both the chilled fruit and *Cercospora*-infected fruit clearly had higher tannin contents in the pulp than the healthy fruit ripened at tropical temperatures. The data for the pulps given in Tables VII and VIII also showed the effect of prolonged storage at 53° F. on the pulp tannin content. Preliminary data, not presented, indicate that the tannin content of the pulps of severely 'gassed' fruit (Wardlaw, 1940) is also higher than normal ripe fruit.

Colour changes in bananas and in tannins. The pulp of bananas from plants severely infected with *Cercospora* leaf-spot is buff, sometimes almost salmon-pink, instead of creamy white as in healthy fruit. The skins of fruits which have been chilled by prolonged exposure to relatively low temperatures (53° F. or lower for Gros Michel bananas) become russeted or bronzed, and may develop dark brown patches.

TABLE IX

Eating Quality and 'active' Tannin Content of Pulp

	Days from cutting.	Eating quality.	Tannin (units per 100 gm. pulp).
<i>Healthy.</i> Standard $\frac{3}{4}$ -full. Ripened at trop. temperatures, 'Sprung' on 11th day, coalescing brown spots on 18th.	14	Good.	0.94
	15		0.97
	16		1.07
	18		0.99
<i>Healthy.</i> Standard $\frac{3}{4}$ -full. Stored at 53° F. throughout experiment.	52	Severely chilled.	2.17
	53	Astringent, with soggy 'break'.	2.14
	54		2.28
<i>Severe Cercospora infection.</i> Standard $\frac{3}{4}$ -full. Ripened at trop. temperatures. 'Sprung' on 2nd day, brown-yellow by 10th.	7	Eatable but astringent. Typical <i>Cercospora</i> fruit.	2.85
	8		2.17
	9		2.12
	10		2.19

When attempting to isolate tannins from banana skins and pulps barium carbonate was added to solutions of the tannins in dilute sulphuric acid. After filtration the residue, consisting of barium sulphate and carbonate, was found to have retained some tannin substances which on exposure to air slowly oxidized. During oxidation, these tannin compounds developed various colours which showed clearly against the white background of the barium salts. The first colour in order of time was buff; this gave place to a salmon-pink, and was then followed by bronze, turning to darker and darker shades of brown until a brownish-black colour finally appeared. The tannins extracted from the banana, therefore, at various levels of oxidation reproduce all the colours observed in bananas which, for various reasons, have ripened anomalously.

IV. DISCUSSION

It has been shown that in the green banana there are two kinds of tannin-containing elements: (a) latex vessels present both in pulp and skin, and (b) small scattered parenchymatous cells in the outer and middle regions of the skin. In such fruit the tannin is in solution and apparently evenly distributed within the vessels and cells. As the banana ripens the nature of the contents of the latex vessels changes; caking or drying-out is observed, and in the skin vessels the formation of oil drops. Simultaneously, tannins in solution appear to some extent in parenchymatous cells, particularly those adjacent to the latex vessels of the skin. The tannin content of the small scattered cells of the skin appears to undergo little or no change.

As the banana ripens its pulp loses its astringent property and it is reasonable to assume that this loss is associated with the observed changes in the tannin contents of the laticiferous vessels. An effort has been made to place these qualitative observations on a more definite basis by devising a method for the estimation of 'active' tannins, i.e. those possessing particular properties such as astringency and, for our purpose, the ability to inhibit the activity of a

diastatic enzyme. The existence of any relation between such a subjective property of a substance as astringency and a definite inhibitory power towards an enzyme must remain an assumption. However, it may be said that if estimation of the inhibitory power of a solution is a measure of its active tannin content and if astringency is a property of the tannin in its active state, then by estimating the solution's inhibitory power we obtain an indication of the intensity of its astringency.

Identity of diastase-inhibitor. Before considering the results of the method of estimating astringent tannins it is necessary to be reasonably sure that the diastase-inhibitor is a tannin substance; it is known that many substances, e.g. metallic salts,¹ sugars, and proteins inhibit diastatic action.

Consideration of the available evidence concerning the substances estimated in aqueous extracts of banana pulp leads to the conclusion that there is a high probability that the inhibitory substance in these extracts is a tannin substance. The following observations may be noted: (1) The green banana contains considerable amounts of soluble tannins which disappear during the ripening of the fruit (Scurti and Pavarino, 1933, and the present work). (2) A decrease in inhibitory activity has been demonstrated for both pulp and skin during ripening. (3) Tannins are removed from solution by hide-powder; in suitable dilution, the inhibiting substance is similarly removed from banana extracts. (4) Hide-powder contains no soluble materials which affect diastatic activity. (5) Solutions of taka-diastase passed through hide-powder retain their full activity. (6) A solution of tannic acid on standing slowly darkens and loses its inhibitory activity; so too an aqueous extract of banana skin or pulp on standing turns brown and loses its inhibitory property.

The quantitative observations presented above are in agreement with the qualitative observations of Scurti and Pavarino (1933) on the effects of excessive cold storage on bananas. These investigators observed leakage of the soluble tannins in such fruit from the tannin-containing vessels into the surrounding tissues, accompanied by discoloration. The present work has shown that the pulp of chilled fruit contains a larger amount of inhibiting substance than does that of normal fruit.

Relation between changes in tannin content and other metabolic changes. The detailed relations between the changes in the amounts of tannin substances and changes in other metabolites remain to be traced. The suggested relation between the appearance of aldehyde² and the disappearance of tannin in the banana during ripening (Scurti and Pavarino, 1933) has yet to be followed quantitatively.

Acetaldehyde is a probable product of the respiration process of the banana fruit under conditions of low oxygen pressure and it is therefore noteworthy

¹ During attempts to isolate a sample of banana tannin, using basic lead acetate, contamination with lead introduced a difficulty into the estimation of crude isolates owing to its inhibitory power.

² Aldehyde has been reported present in the ripe banana (Griebel, 1919, 1924), and preliminary quantitative determinations have detected minute amounts in the pulp of green bananas (Barnell, unpublished).

that the tannin content, particularly of the pulp, falls rapidly during the stage preceding the attainment of the sprung condition, i.e. during the stage in which the internal oxygen concentration is falling (Wardlaw, Leonard, and Barnell, 1939; Wardlaw, 1940). After reaching a minimal value at the sprung condition the internal oxygen concentration usually recovers to some extent, but the tannin content of both pulp and skin continues to fall until the eating-ripe stage, after which an approximately steady concentration is maintained.

The colour changes observed in fruit from *Cercospora*-infected plants and in chilled fruits may possibly be related to changes in the internal atmospheres of the fruits produced by these conditions. There is no information at present available concerning the effect of *Cercospora* infection on the internal atmospheres of bananas, but considerable differences have been observed in the trends of oxygen and carbon-dioxide concentrations in severely chilled fruit from those observed in bananas not subject to this condition (Wardlaw, 1940, Fig. 2). The internal oxygen concentration in chilled fruit does not show such large changes as in fruit subjected to a shorter period at 53° F. It falls relatively slowly, does not reach such low values, and the recovery after the sprung minimum is relatively slight. Any hypothesis based on these few observations must be tentative, but it is apparent that anaerobic respiration may play a smaller part in chilled than in unchilled fruit since the oxygen concentration remains higher in the former, resulting perhaps in a smaller acetaldehyde formation. With less acetaldehyde, a smaller amount of tannin may be rendered non-astringent. Of the remaining tannin, that in the skin may be oxidized, giving rise to the characteristic colours of chilled fruit, while that in the pulp may remain in the free astringent state accounting in some measure for the anomalous flavour and also possibly inhibiting, to some extent, the hydrolysis of starch.

Tannins and latent fungal infections. There is a group of pathogens which can enter unwounded green fruits and produce dormant or latent infections in the superficial tissues but show no further activity until the fruits have become ripe (Wardlaw, Baker, and Crowdy, 1939). Anthracnose spotting does not begin until the tannin concentration is very low, and it is reasonable to suggest the existence of a causal relationship between the tannin concentration of the skin and the activity of these infections. In this connexion Cook and Taubenhaus (1911) showed that the spores of the fungus responsible for anthracnose spotting, *Gloeosporium musarum*, would not germinate in media containing more than 0.6 per cent. of 'tannin'.

SUMMARY

1. The distribution of tannin-bearing elements within the tissues of the Gros Michel banana fruit has been surveyed and the changes in these elements during ripening observed.

2. A tentative method for the estimation of astringent tannins is described. The method depends on the diastase-inhibiting property of 'free' or 'active' tannin in aqueous solution.

3. Approximately four times as much 'active' tannin was found in the skin as in the pulp of the freshly harvested banana. During storage the amount fell in both skin and pulp, particularly in the stage just preceding the attainment by the banana of the 'sprung' condition. Small but approximately constant amounts remain in pulp and skin (about twice as much in the skin as in the pulp) during the eating- and over-ripe stages.

4. The pulp of abnormal ripe fruit (e.g. chilled fruit or fruit from *Cercospora* leaf-spot infected plants) contained a higher amount of 'active' tannin than that of normal fruit.

5. The slow oxidation of crude banana tannins gives rise to colours similar to those observed in the pulp of fruit from *Cercospora*-infected plants and in the skins of chilled fruits.

LITERATURE CITED

- ALLEN, A. H., 1927: Commercial Organic Analysis, vol. v. 5th ed., London.
- BARNELL, H. R., 1940: The Banana in Relation to Human Nutrition. *Trop. Agriculture*, xvii. 8. 143-6.
- 1941: Studies in Tropical Fruits. XI. Carbohydrate Metabolism of the Banana Fruit during Ripening under Tropical Conditions. *Ann. Bot., N.S.*, v. 18. 217-47.
- 1941a: Studies in Tropical Fruits. XIII. Carbohydrate Metabolism of the Banana Fruit during Storage at 53° F. and Ripening at 68° F. *Ann. Bot., N.S.*, v. 20. 607-46.
- 1943: Studies in Tropical Fruits. XIV. Carbohydrate Metabolism of the Banana Fruit during Storage at 53° F. *Ann. Bot., N.S.*, vii. 25. 1-22.
- BERGIUS, 1778: *Materia medica e regio vegetabili*. Stockholmiae. (Quoted by Nierenstein, 1934.)
- BROWN, H. T., and MORRIS, G. H., 1893: A Contribution to the Chemistry and Physiology of Foliage Leaves. *Journ. Chem. Soc.*, lxiii. 604.
- CHAPMAN, A. C., 1907: Hop Tannin. *Journ. Inst. Brewing*, xiii. 646.
- COOK, M. T., and TAUBENHAUS, J. J., 1911: The Relation of Parasitic Fungi to the Contents of the Cells of the Host Plant. I. Toxicity of Tannin. *Delaware Coll. Agric. Expt. Sta. Bull.* 91.
- CREDE, E., 1925: *Journ. Amer. Leather Chem. Assoc.*, xx. 573.
- CULLEN, 1789: *A Treatise of the Materia Medica*, ii. 1-52. Edinburgh.
- GRIEBEL, C., 1919: *Zeit. für Nahrungs- und Genussmittel*, p. 77.
- 1924: *Ibid.*, p. 221.
- HUBERT, H., 1924: Ueber den Zustand und Rolle der Gerbstoffe in der Pflanze. *Jahr. Wiss. Bot.*, lxx. 278.
- LYOYD, F. E., 1911: The Behaviour of Tannins in Persimmons. *Plant World*, xiv. 1.
- 1912: The Occurrence and Functions of Tannin in the Living Cell. *Trans. Roy. Soc. Canada, Ser. III*, xvi, Sect. V, 1.
- MICHEL-DURAND, E., 1928: Recherches physiologique sur les composés tanniques. *Rev. Gen. Bot.*, xl.
- 1929: *Ibid.* xli.
- 1930: *Ibid.* xlii.
- NIERENSTEIN, M., 1934: *The Natural Organic Tannins*. London.
- PHILLIPS, R. C., 1922: *Journ. Amer. Leather Chem. Assoc.*, xvii. 565.
- SCURTI, F., and PAVARINO, G. L., 1933: Sulle Alterazioni Biochimiche de le Banane subiscono nelle Basse Temperature. *R. Staz. Chim.-agricola*, xii.
- SLOCUM, A. H., 1933: Banana Chilling Studies. II. Effect of Low Temperature upon Cell Structure. *Bull. No. 48*. Res. Dept. United Fruit Co.
- TATLOCK, R. R., and THOMPSON, R. T., 1910: Analysis of Tea and Tea Infusions. *Analyst*, xxxv. 103.
- TORO, R. A., 1922: Studies on Banana Anthracnose. *Journ. Dept. Agric. Porto Rico*, vi.

- WARDLAW, C. W., 1937: Banana Diseases. X. Further Observations on Cercospora Leaf Spot of Bananas. *Trop. Agriculture*, xiv. 4. 117-18.
- 1940: Preliminary Observations on the Refrigerated Gas Storage of Gros-Michel Bananas. *Mem.* 15. Low Temp. Res. Sta., I.C.T.A. Trinidad.
- and MCGUIRE, L. P., 1931: Transport and Storage of Bananas with special reference to Chilling. *E.M.B. Report* 45.
- BAKER, R. E. D., and CROWDY, S. H., 1939: Latent Infections in Tropical Fruits. *Trop. Agriculture*, xvi. 12. 275.
- LEONARD, E. R., and BARNELL, H. R., 1939: Studies in Tropical Fruits. VII. Notes on Banana Fruits in Relation to Studies in Metabolism. *Ann. Bot., N.S.*, iii. 12. 845-60.
- LEONARD, E. R., and BARNELL, H. R., 1939a: Metabolic and Storage Investigations on the Banana. *Mem.* 11. Low Temp. Res. Sta., I.C.T.A. Trinidad.
- WILSON, J. H., 1928: *The Chemistry of Leather Manufacture*, 2nd ed., i. 448. New York. (Quoted by Nierenstein, 1934.)

The Distribution of Weight Change in the Young Tomato Plant

I. Dry-weight Changes of the Various Organs

BY

D. W. GOODALL

(*Research Institute of Plant Physiology, Imperial College of Science and Technology,
London, and Experimental and Research Station, Cheshunt*)

With five Figures in the Text

	PAGE
INTRODUCTION	102
REVIEW OF LITERATURE	103
I. Diurnal Course of dry-weight change	103
II. Effect of External Conditions on dry-weight change	104
III. Dry-weight Change of Leaves as affected by Age	105
METHOD OF EXPERIMENTATION	105
I. Selection of Plants	105
(a) Morphology	106
(b) Growth-Rate	106
II. Method of Determining Change in Dry Weight	106
(a) Historical and Critical	106
(b) The Method adopted	107
(c) The Spring Balances	108
(d) The Drying Oven	109
III. Experimental Procedure	111
RESULTS	112
I. Changes in Dry Weight of Plant as a Whole	112
II. Changes in Dry Weight of Organs separately	119
SUMMARY	125
APPENDIX: STATISTICAL ASPECTS OF THE INVESTIGATION	126
I. The Measurements used as Bases for Estimation of Changes in Dry Weight	126
(a) Leaves	126
(b) Stems	128
(c) Cotyledons	128
(d) Roots	129
(e) The Whole Plant	129
II. Estimation by Measurement compared with Estimation by Pairing	130
III. Calculation of the Dry-weight Changes	131
(i) Variation in Regression Coefficients	131
(a) Between Samples	131
(b) Between Experiments	132
(c) Between Organs	133
(ii) Variation in Error Estimates	133
(a) Between Experiments	133
(b) Between Organs	134
(iii) Method of Calculation of Dry-weight Changes	134
ACKNOWLEDGEMENTS	136
LITERATURE CITED	136

INTRODUCTION

THE present research on dry-weight changes in young tomato plants under normal cultural conditions arose as a continuation of the physiological study at the Cheshunt Experimental and Research Station begun in 1930 by Bolas and Melville and continued by these workers and Selman. By the time the writer began work in 1935 a considerable number of experiments had been performed at all times of year to determine the rate of change in dry weight, during the hours of daylight, of the root, the stem, and the leaves taken together. A smaller number of experiments had also been performed during the night. From these observations conclusions had been drawn as to the assimilation and respiration of the plant as a whole, the distribution of the assimilate between the three portions into which the plant had been divided, and the dependence of these processes on the natural fluctuations in environmental conditions.

The further development of this work was seen to lead in two directions. First, to treat all the leaves as one physiological unit was clearly unsatisfactory, for young and old leaves were certainly behaving differently. It appeared desirable to estimate separately the dry-weight changes of the leaves of different age, and if possible to obtain a measure of the amount of translocation to or from each leaf. The latter section of the work will not be dealt with in the present paper. Second, in order to obtain some indication of the diurnal course of these three processes it was desirable to divide the day into rather shorter periods than those employed in the earlier work, and in each experiment to continue observations throughout the 24 hours. This period was accordingly divided into four parts; i.e. the periods of daylight and of darkness were each divided into halves. Such 24-hour experiments were performed on 26 days distributed fairly evenly through the year. Thus information was obtained of the annual course of these phenomena, and of their dependence on environmental conditions as these varied through the year.

The aim in the experiments was to employ always morphologically comparable material. The plants used throughout were of the variety E.S. 1 at the eight-leaf stage, i.e. the eighth leaf had reached the length of about 1 cm. and was the smallest manipulable leaf. The material was thus as similar as possible to that which had been used in the earlier work. The appearance of the plants, however, varied considerably with the time of year, being affected by the environmental conditions of the whole period during which they had been developing. The plant in winter takes nearly 3 months to reach the eight-leaf stage and is then roughly cylindrical in form, all the lower leaves being of about the same size; the cotyledons have usually dropped, and the first foliage leaf is entering the phase of senescence. In summer the eight-leaf plant is only 1 month old and is conical in form, the second being much larger than the later formed foliage leaves; moreover the cotyledons are still green, and the first foliage leaf is still growing. Plants at this morphological

stage are in the summer about to enter upon the flowering phase, the first flower initial having already been formed, while in the winter this does not occur until after about twelve leaves. The results of the experiments thus represent not the response of a 'standard plant' to the particular environmental conditions of the day of experiment, but the response of a plant grown up to a predetermined stage under the conditions prevalent at that time of year.

The experimental plants were under normal conditions of glasshouse culture up to the actual time of the experiments, and until then were in charge of the nursery staff of the station.

REVIEW OF LITERATURE

I. Diurnal course of dry-weight change.

There has been a considerable number of observations on the diurnal course of dry-weight change in leaves, mostly using Sachs' method. The diurnal course of dry-weight change in other organs or in the plant as a whole has hitherto been relatively neglected.

It has fairly often been found that the leaves increased in dry weight during the morning, while after midday the dry weight remained stationary or declined. Müller (1904) found this in most of the plants that he studied, especially in those which stored starch; there was, however, often a further small increase in the evening. Kostytschew et al. (1926) reported a morning increase and afternoon decrease in *Phragmites* and *Lappa*, Tschesnokov and Bazyrina (1930) in *Petasites officinalis* and the pea, Montemartini (1933) in *Chorisia* and *Helianthus*, and Watson (1936) in the potato. Miller (1917) in *Sorghum* and Alexandrov (1928) in the vine found the rate of dry-weight increase more rapid in the morning than in the afternoon, while Denny (1930) reported that *Salvia* leaves continued to increase in dry weight till the middle of the afternoon, and then decreased.

On the other hand, Kostytschew et al. (1926) found that in *Betula* the dry-weight increase was continuous throughout the day. Porter (1937) stated that dry-weight increase in tomato leaves was more rapid in the afternoon than in the morning; and Guttenberg (1927) found that in evergreens the dry weight of the leaves actually decreased during the morning and increased again in the afternoon. Data extracted from Stănescu's (1927) table for *Polyanthus* show the dry weight fairly constant through the morning, rising till the evening, remaining constant until shortly before sunrise, and then suddenly decreasing.

The course of dry-weight change at night has been little studied. Apart from observations over the night as a whole or over a single period of the night (e.g. Sachs, 1884; Watson, 1936), the only figures available appear to be those of Stănescu cited above, and Denny (1933). The latter found that in the stringless bean and the peanut the rate of loss of dry weight fell off continuously through the night; in cutshort bean the rate of loss increased till midnight, but afterwards became negligible.

Melville (1934) found in an experiment with the tomato that the course of dry-weight change of the whole plant during the day followed fairly closely the changes in light intensity.

II. Effect of external conditions on dry-weight change.

Using the leaf-punch method, Mudrack (1935) found the gain in dry weight during the day by leaves of *Ficaria verna* to be much greater in early April than later when the temperature rose above 15° C. The gain was greater in shade than in full sunlight. Apart from this work, observations and experiments on this aspect of the subject have been almost exclusively concerned with changes in the dry weight of the plant as a whole. Some have relied on the natural fluctuations in the environment; Hackenberg (1909), for instance, tried to relate his 'Substanzquotient' values to the light, temperature, and humidity conditions prevailing. Brenchley (1920) found the rate of dry-weight increase of pea plants through the year; the rate during the first 4 weeks of growth was positively correlated with temperature but not with hours of sunshine; thereafter it was positively correlated with both factors. There was a significant negative regression on night temperature.

Gregory (1926) followed the increase in dry weight of barley plants through the season; the net assimilation rate was correlated positively with the mean maximum (day) temperature and with the total radiation, but negatively with the mean minimum (night) temperature. These three factors accounted for over 80 per cent. of the total variation. Williams (1939) made similar observations on oats and found that the net assimilation rate, if calculated on the basis of the protein nitrogen content of the leaves, followed closely the mean maximum temperature.

In the earlier work at Cheshunt on tomatoes a number of observations were carried out on the relation of the rate of dry-weight accumulation in the plant during the day to the light intensity and temperature. Bolas (1934) showed that with increasing light intensity the optimal temperature for the accumulation of dry matter increases from 62° F. at 100 f.c. to > 90° F. when the intensity reaches 1,000 f.c.; conversely, the optimal light intensity increases with the temperature from about 200 f.c. at 60° F. to 950 f.c. at 85° F.

In other work on this subject the environmental conditions have been modified for the purposes of the experiment. Davis and Hoagland (1928) investigated the effect on the yield of wheat of varying the light intensity, the day length, or the temperature, the other two factors being in each case kept constant. With increase in light duration up to 12 hours per day the yield increased exponentially; from 12 to 24 hours the increase was linear. With increasing light intensity the increase was linear, while the optimum temperature was found to be 25° C. Shirley (1929) in shading experiments found that up to an intensity of 700 f.c. the amount of dry matter formed was proportional to the light intensity.

Arthur et al. (1930), as part of an elaborate investigation, found for the tomato that the optimal day length for dry-weight increase depended on

the light intensity. At 450 f.c. the optimal day length was 17 hours; at 800 f.c. 12 hours. Continuous illumination caused necrosis and death. A combination of 800 f.c. and 78° F. gave greater dry-weight increase than 1,200 f.c. and 68° F.

Ashby and Oxley (1935), working with *Lemna*, found that the net assimilation rate varied linearly with the light intensity (up to 1,600 f.c., the highest intensity used); above 18° C., it was independent of temperature.

Novikov (1936) grew cotton plants in chambers with reduced light intensity and found that those receiving 21 per cent. of daylight grew 32 per cent. better than those in full daylight. In similar experiments with the tomato Porter (1937) found that plants receiving 22.3 per cent. of full sunlight attained a final dry weight 70.5 per cent. of that of plants under full illumination; this means a difference in the daily relative growth-rate of only 0.5 per cent. The same author found that the rate of dry-weight increase of leaves during the day (by Sachs' method) is correlated positively with light intensity and temperature, but negatively with humidity.

Smith (1933) found that the optimal day length for dry-weight accumulation varied with the age of the plant. For young plants of various species the optimum was 24 hours, while for older plants it was shorter. Roodenburg (1937) in experiments on the cucumber concluded that neither the intensity nor duration alone but the amount of light energy received was of importance in determining growth.

Melville (1937) showed that in the tomato lengthening the night beyond a certain point (17 hours in one experiment, less in another) reduced the rate of dry-weight accumulation on the following day.

III. Dry-weight change of leaves as affected by age.

Alexandrov (1924) measured the dry-weight change in sunflower leaves of different ages by Sachs' method; the maximum increase, he found, was in the fourth leaf below the highest one tested. Eidelman and Bankul (1933*a*) in the same way found the dry-weight changes in the different leaves of spring wheat at various stages of development; at the 'shooting' stage, for instance, the sixth leaf was increasing most rapidly. Denny (1930) measured the dry-weight changes of three consecutive pairs of leaves of *Salvia* by the twin-leaf method. He found very little difference in rate of dry-weight increase during the day, though the youngest leaf lost less in dry weight than the others during the night. Melville (1934) found that in the tomato at the nine-leaf stage the highest rate of dry-weight increase occurred in the eighth leaf.

METHOD OF EXPERIMENTATION

I. Selection of plants.

It was obviously desirable to reduce if possible the variability of the plants sampled by eliminating any abnormal plants before the experiment, and by suitable selection from the apparently normal remainder. This selection was based on two characteristics: morphology and growth-rate.

(a) *Morphology.* In most batches of tomato seedlings the majority of plants have a regular spiral phyllotaxy. In some plants, however, the spiral phyllotaxy does not appear till after the third leaf; the first two leaves are very similar, and are either opposite or in the reverse positions from those which would accord with the subsequent phyllotaxy. Such plants will be referred to as 'opposite'; their frequency in the batch increases with the age of the seed (Goodall, 1938). When these 'opposite' plants were compared with the more normal 'alternate' ones in the same batch it was found that they differed in the size of leaves at the same level, and also in the relation of the dry weight of the leaves to their length. On this account it was decided that it would be better not to use the 'opposite' plants in these experiments.

(b) *Growth-rate.* It was evident that selection on the basis of growth-rate and size would tend to eliminate abnormal plants, such as any with latent mosaic disease, and would thus reduce the sampling errors. Accordingly the following procedure was adopted. Immediately before potting, the seedlings in the seed-boxes (of which 6, containing in all 270 seedlings, were sown on each occasion) were inspected, and any obviously 'opposite' plants were rejected, together with any departing widely from the average size. About 150 plants remained. After some weeks, when the fifth leaf was unfolding, the plants were again inspected, and any 'opposite' plants that had been overlooked were removed; of the remainder, about 100, chosen on a basis of uniformity of size and colour, were transferred to the glasshouse chamber in which the experiments were carried out. Finally, there was a third selection in respect of size one or two weeks later when the plants were ready for the experiment. This left the requisite 72 plants. By these three successive selections for size, not only was fairly uniform size ensured, but also the elimination of any plants with abnormal growth-rate during the preceding weeks.

II. Method of determining change in dry weight.

(a) *Historical and critical.* With an unlimited supply of plant material and of labour, doubtless the most satisfactory method of determining dry-weight changes in plants and their parts is by random sampling. However, for most investigations in which the change over a short period is required the error can only be reduced to a reasonable figure if a very large number of plants is used. Various devices have therefore been adopted to reduce the variation between the initial weights of the initial and final samples.

The first and probably best known of these devices was the *half-leaf method* of Sachs (1884). This depended on the fact that the two halves of a leaf about the midrib are usually more similar in weight than are two separate leaves. At the beginning of the experiment one half of the lamina was separated from the midrib, and a definite area was cut out of the detached half, using a template and avoiding the larger veins. This piece of definite area was dried. At the end of the experimental period the other half was treated in the same way, and the difference in dry weight between the two pieces

divided by the area of the template gave the dry-weight increase per unit area. The attached half of a leaf could remain healthy for months.

Thoday (1909) pointed out a serious source of error in this method; for during the course of the experimental period the area of the leaf was liable to change in daytime by as much as 5 per cent. Montemartini (1930) found even greater changes in area, up to 18 per cent. during the course of the day. Thoday showed that this source of error could be avoided if the area to be sampled was marked on both halves of the leaf at the outset by a rubber stamp. With care, this source of error between the two halves could be reduced to 1.5 per cent.

It is, however, unsafe to regard the changes taking place in any mutilated leaf as a measure of the changes taking place when intact. Apart from the effect on respiration loss of injury and mutilation (Johnstone, 1925; Harris, 1929; Audus, 1939), Combes (1928, 1928*a*) found increase in nitrogen translocation and in dry-weight loss. On the other hand, Eidelman and Bankul (1933), and Stcheglova and Tschernyscheva (1933) reported an increase in assimilation of the remainder following removal of part of the lamina, and Lubimenko (1923) in *Raphanus* leaves found a greater rate of increase in dry weight when 25 per cent. had been removed during unfolding.

Another method of reducing the error of dry-weight change measurements with a limited number of leaves is the *twin-leaf method*; this was first suggested, though not used, by Sachs (1884) and was studied in some detail by Denny (1930). In this method, of two opposite leaves or leaflets one is sampled at the beginning of the experimental period, the other at the end. In the three species used Denny found that the mean deviation between the dry weights of opposite leaves sampled simultaneously lay between 4 and 6 per cent. Watson (1936) found that the error using the twin-leaf method for potato leaflets was 11.9 per cent., whereas by direct sampling it was 59.3 per cent. According to Palmquist (1938) the mean difference between the dry weights of twin leaflets in the bean is less than 1 per cent. Though to less extent than in the half-leaf method, there is still the risk that the mutilation will affect the plant's behaviour.

Finally there is the method used in the earlier work in this series and described by Bolas and Melville (1933), which bears certain resemblances to the twin-leaf method. For these experiments '*paired plants*' were used. Pairs of plants were selected by eye for close similarity in respect of height, stem thickness, and leaf area; of each pair one plant was sampled at the beginning of the experimental period and the other at the end. The standard deviation of the changes in the total leaf dry weight found by this method varied from 10 to 20 per cent. of the initial dry weight; that for the dry weight of the whole plant was rather less. Though these errors were rather large, this method had the great advantage that the plants used were fully normal, and that their behaviour was not affected by the experimental treatment.

(b) *The method adopted.* The paired-plant method was not primarily intended for finding the changes in dry weight of individual leaves. Melville,

however, on one occasion (1934) made an attempt to use it for this purpose, comparing the plants in much greater detail than in the main experiments. The coefficients of variability of the changes ranged from 9.6 per cent. in the fourth leaf to 160 per cent. in the eighth leaf. The low precision of this method in determining changes in the smaller leaves weighed against its adoption. Moreover, the method involved the rejection of some 80 per cent. of the plants available, and involved considerable labour in the selection of the pairs.

Thus it became necessary to find some method of correction for the unavoidable differences between the members of each pair.

It seemed that such a correction could best be applied by taking some suitable measurement of the plants in each sample at the outset of the experiment, and subsequently adjusting the dry weights found for the initial and final samples in accordance with the initial differences in measurement. A full account of this process is given in the Appendix, together with a comparison of the errors involved in this method of estimation with those of the 'pairing' method. These errors show that the correction of dry-weight changes by the initial measurements of leaf and stem length is a substantial improvement on the 'pairing' method applied to individual organs, particularly in respect of the young leaves. The errors encountered in this method approach those found by Denny (1930) for the twin-leaf method and it involves no mutilation of the plant.

The leaves were measured from the axil to the tip, and on sampling the leaf was separated from the stem at the axil. This procedure was chosen because it was found to give the closest relation between measurement and weight. It involved, however, a departure from the practice in the earlier work in this series in which the leaf had been separated at the lowest leaflets, the petiole being included with the stem.

(c) *The spring balances.* The method of determining dry-weight changes involved the weighing, separately and individually, of a large number of plant parts. In fact, in each of the main experiments there were 792 organs to be weighed. For this large number balances suitable for rapid use without a very high degree of accuracy were desirable. Therefore spring balances of the Joly type were constructed with suitable ranges. These were a modification of those described by Bolas and Melville (1933). Four springs of different sizes were wound from phosphor-bronze wire extended almost to breaking-point, and suspended in wooden tubes above a pan chamber fitted with a sliding glass front.

Air temperature was found to affect the extension very little, but the zero reading might be altered by changes in temperature; accordingly an adjustment for the zero was obtained by suspending the spring from a silk thread wound on a rod rotated by a worm gear. The zero was checked, and adjusted if necessary, after each 10 or 20 weighings.

The design for the pans was an improvement on that used by Bolas and Melville, only a single support being used so that the material could more readily be placed on and removed from the pan; the framework was of copper

or steel wire, and the pan was covered with aluminium foil reduced in thickness in the case of the smallest balance by acid treatment. The pan was not suspended directly from the spring but by a short link of wire to the centre of which a small metal disc was soldered. A strip of mirror was fixed to the back of the pan chamber, beside the scale; in weighing, the disc already mentioned was alined with its image in the mirror to eliminate parallax. In order to reduce weight the parallax disc was omitted from the construction of the smallest balance, and the junction of the pan with the spring was used instead.

To the top of the pan support was attached a couple of hooks, used in fresh-weight determinations, the results of which, however, are not discussed in the present paper. The fresh leaf was hung by its basal leaflets from these hooks, obviating the necessity for balancing it precariously on a much smaller surface or folding it with the risk of loss of weight. For drying the larger leaves were lightly folded to save space.

The speed of weighing is greatly reduced by waiting for an oscillating spring to come to rest, so the vibration was 'damped' in the largest balance by a wire with a horizontal metal disc at the end attached to the bottom of the pan and immersed in a cylinder of water below the pan chamber. In the case of the smaller balances rigidity combined with sufficient lightness could not be obtained by using wire, so a fine glass rod was employed terminating in a bulb partly filled with water, the weight of which when submerged was negligible; in these balances after the object had been placed on the pan the latter was drawn to the bottom of the scale and allowed to rise, thus ensuring constant wetting of the rod. It was found necessary to add a little antiseptic to the water to prevent the growth of bacteria. In the case of the smallest balance no water 'damping' was required as the air in the pan chamber, when the sliding door was closed, sufficiently 'damped' its vibration. The smallest balance would carry 15 mg., and was graduated in units of 0.1 mg., while the maximum load of the largest was 3 gm. and its graduation 0.05 gm.

(d) *The drying oven.* For drying a large mass of plant material to determine dry-weight changes, the ordinary type of drying oven, if heated from below and at the sides, is unsuitable unless the material has first been killed, since some time elapses before the temperature in the middle of the oven reaches a level high enough to stop respiration. Bolas and Melville (1933) designed a vacuum oven with electrical heating elements in the shelves; this arrangement equalized the temperature throughout the oven and killed the material soon after it was put in. It was not large enough, however, to enable all the leaves of the plants used in the present experiments to be spread out, so that it became necessary to construct an oven of greater capacity. This was made from a 40-gallon galvanized iron water tank, measuring 26 in. \times 19 in. \times 19 in. In each end 45 circular holes, $2\frac{1}{2}$ in. diameter, were bored; lengths of commercial gutter-pipe, the seams of which had been filled with solder, were placed through the holes and were then soldered in. The inside

of each end of the tank was then filled with a layer of plaster of Paris about 1 in. deep, to safeguard against any small leaks in the soldered joints, and the interior was then coated with bitumen paint. The tank was filled with water, and after a lid of galvanized sheet had been fitted, the whole was covered with a lagging jacket of asbestos boards. It was heated by a gas ring, which was found to maintain the oven at a temperature of $70^{\circ} \pm 2^{\circ}$ C. without a thermostat. It was necessary to add water about twice a week. With leaves

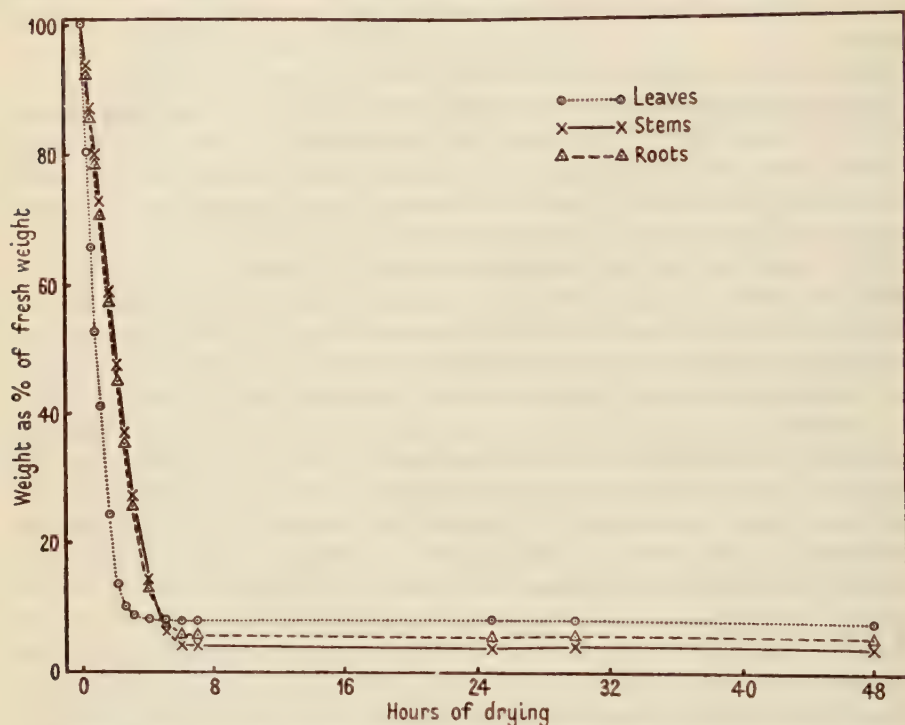


FIG. 1. Curve showing rate of drying in oven of various organs of the plant.

and roots in the oven constant weight was reached in 6 hours, and even in the bulky stems drying was complete in 8 hours (Fig. 1). In practice, the material was always dried for at least 24 hours before weighing.

The plant material was placed for drying in semi-cylindrical trays, 24 in. long, made of perforated zinc sheet with a lining of brown paper. One such tray was used for each plant, the various organs being laid along its length. Thus in the oven the plant material was separated from the hot water by only two layers of metal and one of brown paper—a total distance of less than $\frac{1}{4}$ in.—and very quickly reached the temperature of the oven.

Since there were only 45 tubes in the oven and the number of plants sampled in each experiment was 72, it was necessary to remove some of the early samples to make room for the later ones. Trays that were so removed were later given the full 24 hours' drying before the material was weighed.

III. Experimental procedure.

The 72 plants selected as described above (pp. 105-6) were divided into 9 groups of 8. Of these 5 were for dry-weight change determinations, while the remaining 4 were intended for simultaneous translocation determinations by a method to be described in a subsequent paper. In allotting the plants to these groups an attempt was made to maintain the mean size of the different groups as similar as possible, the index of plant size used for this purpose, as for selection, being the length of the representative fifth leaf. This was designed to reduce the correction of the estimated dry-weight changes for initial differences in size; it was not realized at the time, however, that this procedure, by reducing the variance between the batches, increased that within the batches from which the error variance is derived, and so artificially reduced the significance of any differences between the batches.

The separation of the plants into batches was completed on the afternoon preceding the day of the experiment, and a thermohygrograph was then set up in the greenhouse. The following morning, about 2 hours before sunrise, measurement began. In two of the batches intended for dry-weight change determinations the lengths of the leaves and stem of each plant were measured and recorded; with two workers this occupied 30 to 45 minutes. One of the batches was then taken as an initial sample; the leaves were cut off, rolled, and placed in the drying trays, while the stem and root were separated at the insertion of the cotyledons, slit up the middle, and placed in the trays, one for each plant. When four trays were ready, about 10 minutes after the beginning of this operation, they were taken to the laboratory and placed in the oven; the sampling of the batch was then completed. This operation was so timed that the midpoint fell at dawn. This was taken arbitrarily as the legal time of extinguishing street lamps, i.e. half an hour before sunrise in winter, and one hour before in summer; at this time the illumination in the greenhouse was about 0.01 foot-candle. Dawn was chosen rather than sunrise as the time for beginning the experiment in view of the frequent observations of assimilation in twilight (Kostytschew, 1921*a*; Kostytschew et al., 1926; Heinicke and Childers, 1937). During the day, observations of the light intensity on the greenhouse bench were made at half-hourly intervals by means of a Holophane lumeter, subject to an error of about 7 per cent.

About an hour before noon measurement was again begun. The second batch, which had already been measured once before dawn, was measured again, and also a third batch. The second batch was then divided and dried, this operation having its midpoint at noon (Greenwich time). This process of measurement and sampling was repeated at dusk (lighting-up time) and at midnight. At dawn on the second day the final batch was sampled, the thermohygrograph record was removed, and the experiment was completed. Weighing of the dried organs was begun on the following day; this could be completed in 2 days, though often it was prolonged over a week.

A deviation of 5 or 10 minutes was not uncommon in the times of sampling.

Apart from this, the only exceptions to the above procedure were: (1) in the first experiment (Dec. 7, 1936) no records of temperature or humidity were kept; (2) the experiment of Aug. 23, 1937, had to be abandoned at dusk; (3) the experiment of Oct. 21, 1937, was not begun until noon. In all, 26 experiments of this kind were carried out.

RESULTS

Table I gives details of the environmental conditions in the greenhouse during each experiment. The mean temperature and saturation deficit figures are derived from the thermohygrograph charts; the mean light intensities are calculated from the lumeter readings; in addition the durations of the various experimental periods are listed.

I. Changes in dry weight of plant as a whole.

Table II gives the mean rates of percentage change in dry weight per hour for the plant as a whole, together with the standard errors of these rates of change; the method of calculation is described in the Appendix. The values are given separately for each of the four periods of the day. At the foot of each column are given the mean rates of change for all the experiments, and also separately for those conducted in summer and in winter, the two seasons being taken as separated at the equinoxes.

The rates of change in dry weight over the whole 24 hours, expressed as the relative growth-rate are shown in Fig. 2 B. They range from a negative value on a single winter day to 24.8 per cent. in the height of summer. Five out of the twenty-four values are above +20 per cent. and the average for all the experiments carried out during the six months of summer is +18.8 per cent.

The tomato plant under the conditions of these experiments is thus growing more rapidly than most other plants for which data are available in the literature. These values are listed in Table III.

It is to be doubted whether the maximal value in the present experiments (24.8 per cent.) may directly be compared with the values tabulated above, for whereas the former was determined during a single day, most of the other determinations were made over a week or more. Moreover, the method of experimentation of most of these investigators involved taking plants sown simultaneously, and determining their growth-rates at various times through the season; whereas in the present work the stage at which the plants were used was the same at all times of the year, and moreover one at which the relative growth-rate might be expected to be high. In the development of plants sown in midwinter this stage occurs in early spring when conditions are not as favourable for rapid growth as at midsummer. Even if the summer average of 18.8 per cent. is taken to compare with the values for other plants, however, it stands high on the list, being exceeded only by three other values obtained under natural conditions. The very high value obtained by Sande-Bakhuyzen (1937) was based on a determination made over a period of only 2 days; also

TABLE I
Duration of Experimental Periods and Environmental Conditions

Date of experiment	Dawn to Midday			Midday to Dusk			Dusk to Midnight			Midnight to Dawn		
	Duration (hr.)	Mean light intensity (f.c.)	Mean temperature (°C.)	Mean saturation deficit (mm.Hg)	Duration (hr.)	Mean light intensity (f.c.)	Mean temperature (°C.)	Mean saturation deficit (mm.Hg)	Duration (hr.)	Mean temperature (°C.)	Mean saturation deficit (mm.Hg)	Duration (hr.)
Dec. 7, 1936	4.5	84	—	—	4.4	45	—	—	7.5	—	—	7.7
Dec. 20, 1936	4.6	57	18.5	3.8	4.7	65	21.7	5.8	6.9	18.9	5.2	7.8
Jan. 11, 1937	4.3	61	16.7	4.9	5.1	63	18.3	5.3	7.3	15.6	3.5	7.4
Jan. 18, 1937	5.1	35	11.7	1.6	4.8	83	14.4	2.7	7.3	12.8	2.7	7.8
Feb. 4, 1937	5.1	102	17.8	—	5.7	87	20.0	—	6.6	16.1	—	6.8
Feb. 15, 1937	5.4	146	18.9	4.9	5.3	246	20.6	6.2	6.7	17.8	3.9	7.0
Feb. 23, 1937	5.5	139	10.0	2.1	5.9	141	19.6	4.1	6.0	12.8	2.8	6.6
Mar. 8, 1937	6.0	135	10.8	3.6	6.6	196	15.3	4.9	5.7	10.0	2.5	5.7
Mar. 23, 1937	6.1	470	12.5	4.5	6.8	304	17.2	6.0	5.3	10.2	2.6	5.5
Apr. 5, 1937	6.8	263	15.2	4.3	7.1	246	20.7	7.7	4.8	14.6	3.0	5.2
Apr. 12, 1937	7.3	575	15.8	6.0	7.2	267	20.7	7.2	4.8	13.9	3.3	5.4
Apr. 28, 1937	8.0	62	11.6	1.7	8.2	63	13.9	2.9	3.7	11.1	1.9	4.0
May 6, 1937	8.7	877	16.7	11.0	8.4	333	20.6	10.0	3.5	15.0	2.1	3.4
May 24, 1937	8.8	695	24.4	14.4	9.1	366	24.1	11.0	3.1	17.2	2.8	3.0
June 2, 1937	8.8	690	15.9	5.5	8.9	229	21.7	11.0	3.1	13.0	1.2	3.2
July 7, 1937	9.0	381	20.3	7.8	9.0	139	18.4	3.8	2.9	12.4	0.8	3.1
Aug. 17, 1937	8.1	435	18.3	4.6	8.2	437	23.9	8.6	3.7	16.6	1.5	4.0
Aug. 25, 1937	8.1	273	15.3	4.5	7.4	336	23.9	9.6	—	—	—	—
Sept. 2, 1937	7.6	289	17.2	5.2	7.6	221	16.6	5.5	4.1	17.2	2.0	4.6
Sept. 10, 1937	7.5	592	12.8	5.3	7.1	176	16.6	5.1	4.5	8.6	1.5	4.9
Sept. 27, 1937	7.1	223	20.3	6.0	6.7	207	24.2	10.8	5.3	17.4	3.4	5.0
Oct. 11, 1937	5.6	244	14.7	7.6	5.4	159	10.2	4.0	6.3	16.2	3.0	6.3
Oct. 21, 1937	—	—	—	—	5.4	79	18.6	3.5	6.5	15.7	2.1	6.4
Nov. 11, 1937	5.1	124	12.0	2.3	4.9	100	13.9	2.7	7.1	10.7	1.9	6.9
Nov. 25, 1937	5.0	46	11.1	2.5	4.4	67	13.5	3.4	8.4	10.8	2.7	6.2
Dec. 7, 1937	4.4	42	10.6	2.1	4.3	75	11.0	2.5	7.8	9.6	2.2	7.0
Mean (winter)	5.2	111	13.6	3.5	5.3	115	17.5	4.3	6.8	14.2	2.8	6.8
Mean (summer)	7.9	467	16.2	6.2	7.9	260	20.2	7.4	3.9	13.6	2.1	4.2
Mean (all expts.)	6.5	282	14.8	4.8	6.5	185	18.8	5.8	5.5	14.0	2.5	5.6

TABLE II *Change in Dry Weight (per cent. per hour) of Whole Plant*

Date of experiment	Dawn-midday		Midday-dusk		Dusk-midnight		Midnight-dawn	
		S.E.		S.E.		S.E.		S.E.
Dec. 7, 1936	.	+1.00 0.94	-0.20 0.94		+0.24 0.56		-2.11 0.54	
Dec. 29, 1936	.	+1.55 1.10	+0.04 1.06		+0.14 0.72		-0.04 0.63	
Jan. 11, 1937	.	+0.98 1.29	+0.85 1.10		-0.16 0.76		+0.25 0.75	
Jan. 18, 1937	.	+0.96 0.80	+1.15 0.87		-0.61 0.56		+0.38 0.53	
Feb. 4, 1937	.	+1.88 0.85	+1.39 0.78		-1.47 0.67		-0.21 0.64	
Feb. 15, 1937	.	+1.30 0.76	+1.71 0.74		-0.14 0.62		-0.83 0.62	
Feb. 23, 1937	.	+1.28 0.81	+0.29 0.75		+1.00 0.74		-1.16 0.68	
Mar. 8, 1937	.	-0.14 0.66	+1.94 0.57		-0.52 0.67		+0.85 0.74	
Mar. 23, 1937	.	+1.26 0.54	+1.47 0.46		-0.96 0.55		+0.70 0.55	
Apr. 5, 1937	.	+0.85 0.68	+1.48 0.65		+0.09 0.98		-0.42 0.90	
Apr. 12, 1937	.	+1.72 0.70	+1.48 0.72		-1.35 1.09		+0.11 0.99	
Apr. 28, 1937	.	+0.24 0.41	+0.71 0.40		-0.60 0.88		+0.32 0.83	
May 6, 1937	.	+1.63 0.33	+1.18 0.31		-0.57 0.78		-0.17 0.79	
May 24, 1937	.	+2.02 0.40	+0.91 0.38		-0.11 1.17		-0.21 1.15	
June 2, 1937	.	+2.02 0.40	+0.30 0.40		-0.94 1.14		+0.36 1.11	
July 7, 1937	.	+1.77 0.34	+0.50 0.35		-1.85 1.06		+2.80 1.03	
Aug. 17, 1937	.	+1.03 0.66	+1.39 0.65		-2.30 1.46		+1.58 1.36	
Aug. 25, 1937	.	+1.79 0.36	+1.10 0.40		— —		— —	
Sept. 2, 1937	.	+1.74 0.58	+1.62 0.57		-2.00 1.08		+1.17 0.96	
Sept. 10, 1937	.	+2.26 0.64	+1.20 0.67		-0.46 1.07		+0.16 0.99	
Sept. 27, 1937	.	+0.98 0.57	+0.99 0.59		+0.14 0.77		-0.35 0.80	
Oct. 11, 1937	.	+1.48 0.72	+1.24 0.76		-0.01 0.64		+1.10 0.64	
Oct. 21, 1937	.	— —	+2.12 0.53		-0.37 0.44		+0.11 0.45	
Nov. 11, 1937	.	+1.17 0.51	+0.20 1.06		+0.53 0.73		+0.15 0.75	
Nov. 25, 1937	.	-0.05 0.77	+0.81 0.88		+0.43 0.47		-0.78 0.63	
Dec. 7, 1937	.	+0.32 0.88	+0.02 0.89		+0.37 0.50		-0.02 0.55	
Mean (winter)	.	+0.98 0.23	+0.90 0.22		-0.03 0.17		-0.19 0.08	
Mean (summer)	.	+1.53 0.15	+1.11 0.15		-1.00 0.31		+0.58 0.30	
Mean (all expts.)	.	+1.24 0.14	+1.00 0.14		-0.46 0.17		+0.15 0.08	

Note. The figures given for the standard error are maximum values (see Appendix, p. 130).

TABLE III

Maximal Values for Relative Growth-rate ($r \times 100$) found by Various Authors

<i>Avena sativa</i>	7.8	Wolff, quoted by Blackman (1919)
<i>Triticum vulgare</i>	8.0	Ballard and Petrie (1936)
<i>Gossypium</i> sp.	10.1	Heath (1937)
<i>Beta vulgaris</i> (mangold)	10.4	Watson and Baptiste (1937)
<i>Pisum sativum</i>	11.0	Brenchley (1920)
<i>Beta vulgaris</i> (sugarbeet)	11.5	Watson and Baptiste (1937)
<i>Hordeum vulgare</i>	11.7	Gregory (1926)
<i>Gossypium</i> sp.	12.9	Inamdar et al. (1925)
<i>Cannabis sativa</i>	13.2	Hackenberg (1909)
<i>Avena sativa</i>	14.0	Williams (1936)
<i>Cannabis gigantea</i>	14.7	Hackenberg (1909)
<i>Zea Mays</i>	15.0	Kreusler et al. (1879), quoted by Briggs et al. (1920)
<i>Nicotiana Tabacum</i>	15.5	Petrie et al. (1939)
<i>Helianthus annuus</i>	17.6	Gressler (1907), quoted by Blackman (1919)
<i>Sinapis alba</i>	18.1	Hornberger (1885), quoted by Kidd et al. (1920)
<i>Andropogon sudanensis</i>	19.0	Ballard and Petrie (1936)
<i>Helianthus macrophyllus</i>		
<i>giganteus</i>	19.5	Gressler (1907), quoted by Kidd et al. (1920)
<i>Nicotiana Tabacum</i>	20.5	Kiltz (1908), quoted by Blackman (1919)
<i>Triticum vulgare</i>	28.8	Sande-Bakhuyzen (1937).

the plants were under conditions of continuous artificial illumination, and only the tops were harvested.

For comparison with the relative growth-rate on the day of the experiment,

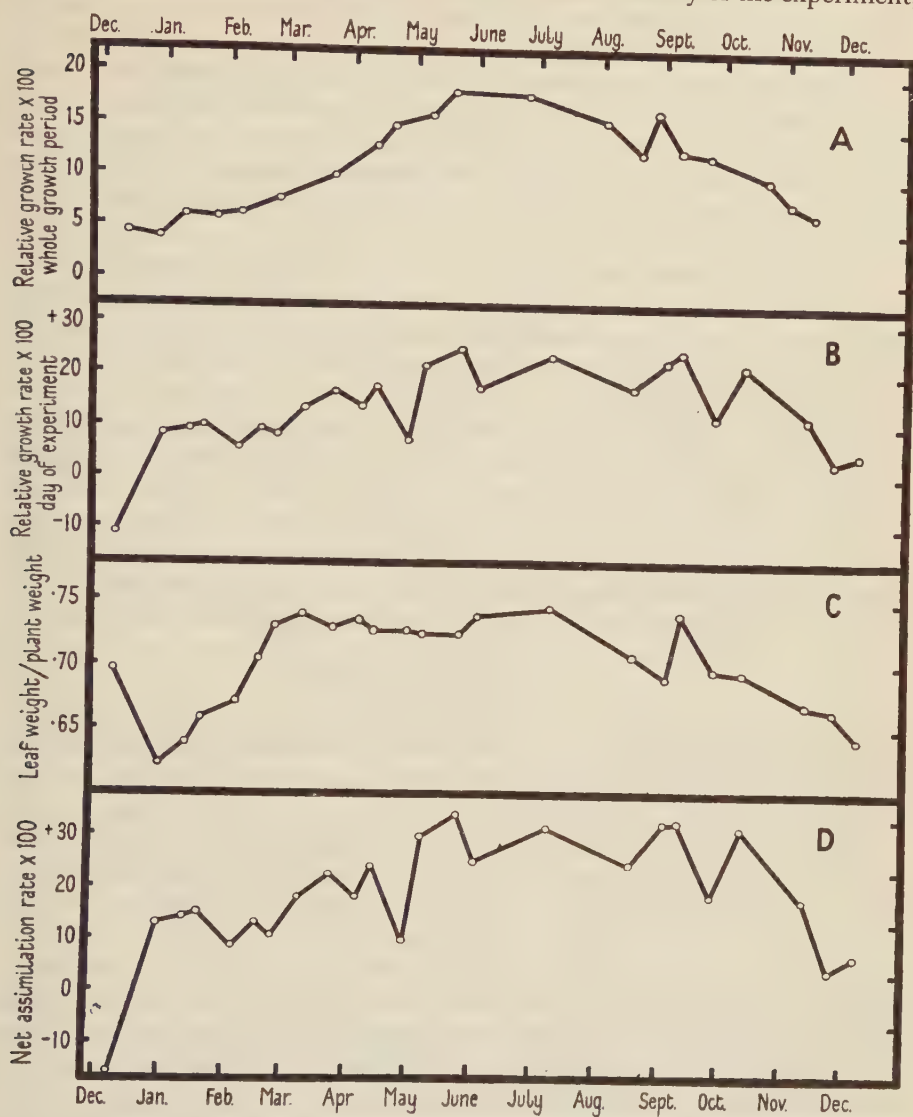


FIG. 2. Relative growth-rate ($r \times 100$) for the whole growing period (curve A) and for the day of experiment (curve B), leaf-weight ratio (curve C), and net assimilation rate ($r \times 100$) on leaf-weight basis (curve D).

in Fig. 2 A are shown the average growth-rates calculated over the whole period from the date of sowing to the conclusion of the experiment, which varied at different times of year from 4 to 11 weeks, and based on the mean dry weight of seed of this variety (2.63 mg.). The figures are, of course, only

approximate, but they accord fairly well with the growth-rates of the experimental days. They are on average rather lower, but since they do not allow for the time taken by the plants to germinate and emerge from the soil, it appears that at some intermediate stage the relative growth-rate may have been rather higher than at the eight-leaf stage. This would be in accord with the results of Sande-Bakhuyzen (1937) with wheat, who found that the relative growth-rate decreased continuously after the period of germination had been passed. These figures for the mean relative growth-rate over the whole period of development naturally show a much more regular seasonal drift than those for the single day of experiment, since they average the increases in dry weight over some weeks.

It is generally recognized, since the work of West et al. (1920), that the relative growth-rate can be analysed into two constituent quantities, namely the leaf ratio F (the proportion of leaf area or dry weight to the dry weight of the whole plant, called structural efficiency by Sande-Bakhuyzen (1937)) and the net assimilation rate E (rate of growth of whole plant per unit of leaf material; also called unit leaf rate). Ballard and Petrie (1936) have represented these by the symbols F_W , F_A and E_W , E_A , where the suffixes indicate whether the leaf dry weight or the leaf area has been used. This analysis has been carried out on the figures obtained in the present work, and the results are presented in Fig. 2 C and 2 D. The leaf-weight ratio given is the mean of the ratios for the two dawn samples; the difference between the two was always small. The net assimilation rate was calculated according to the usual formula:

$$E_W = \frac{(W_2 - W_1)(\log_e L_2 - \log_e L_1)}{(L_2 - L_1)(t_2 - t_1)},$$

where W_1 and W_2 are the dry weights of the whole plant, L_1 and L_2 the dry weights of the leaves, and t_1 and t_2 the times at the beginning and end of the experiment.

The leaf-weight ratio (leaf weight/plant weight) shows a very definite seasonal trend from a minimum of 0.623 at midwinter to 0.745 at midsummer. These values are rather higher than most of the published figures, many of which refer of course to older, often mature, plants. According to Sande-Bakhuyzen's (1937) calculations from Hornberger's (1882) data, for instance, the highest leaf-weight ratio reached by maize is 0.568. The highest value found by Inamdar et al. (1926) in cotton was 0.618. Williams (1936) working with oats gives a maximum of 0.561, while Ballard and Petrie (1936) find for wheat 0.526 and for Sudan grass 0.460. For tobacco, Petrie et al. (1939) give a maximum of 0.78, higher even than the present values for tomato. The high leaf-weight ratio in the young tomato plant doubtless accounts, at least in part, for the high values found for the relative growth-rate. The difference between the winter and summer values of the ratio likewise contributes to the considerable difference in the mean relative growth-rate during these two periods.

Together with the seasonal changes in the leaf-weight ratio, the values of

net assimilation rate display even greater differences between summer and winter. The means are respectively 25.9 and 11.7 gm. per 100 gm. leaf dry matter per day, and the maximum value for any one experiment is 34.3. The data in the literature for net assimilation rate on a leaf-weight basis are not numerous; Hornberger (1882), quoted by Sande-Bakhuyzen (1937), found a maximum value for maize of 17.8, Williams (1936) for oats 24.9, Ballard and Petrie (1936) for wheat and Sudan grass respectively 19.4 and 52.5, and Petrie et al. (1939) for tobacco 32. The figures for tomato are thus not exceptionally high, and confirm the assumption above that the high relative growth-rate at this stage of development is to be ascribed mainly to the high leaf-weight ratio or to the low weight per unit area of the leaf. The present results on the net assimilation rate are not strictly comparable with the earlier work in this series (Bolas et al., 1938), for though the earlier results were expressed on a leaf-weight basis, the experiments were carried out over a 7-hour light period only. Over this period the mean increases, as percentages of the initial leaf dry weight, were in the winter experiments 15.9 and in summer 27.7, with a maximum of 44.4. That these figures are rather higher than the corresponding ones in the present work is not surprising since, in addition to the restricted period of measurement in the earlier work, the petioles were not included in the leaf weight.

Since the leaf areas were not measured in any of the experiments, accurate estimation of the net assimilation rate on an area basis was not possible. However, full data on the lengths of the leaves are available, and a limited number of separate observations have been made on the fairly constant relation between leaf area and leaf length. By use of these figures it has been possible to obtain an approximate estimate of the net assimilation rate on an area basis. The total leaf area was calculated for the initial samples in those experiments in which the plant dry weight corresponded most nearly to the mean values for summer and for winter. Leaf weight/area ratios were derived from this and were used to convert the mean net assimilation rates for winter and summer to an area basis. The results were:

Winter	.	.	.	0.117 gm./sq.dm./week
Summer	.	.	.	0.481 " " "

Heath and Gregory (1938) have tabulated the net assimilation rates found by different investigators for various species of plants and comment on the small range of variation among them. It is interesting to note that, while the present winter mean is very much lower than the values they list, the summer mean value is well within the stated range, falling indeed a little below the mean (0.551).

The net assimilation rate shows great variation among the experiments and the question arises as to what extent this variation can be ascribed to differences in the environmental factors measured. A multiple regression of net assimilation rate on mean light intensity during the day (L), light duration (D), mean day temperature (T), mean night temperature (N), and mean

saturation deficit of the atmosphere (S) has been calculated. For this purpose, however, there have been used not the figures for the net assimilation rate, but those for the dry-weight increase of the plant as a percentage of the initial leaf dry weight. The regression equation is:

$$E_W = 20.0 + 0.0458L + 0.999D + 0.293T + 0.680N - 1.880S.$$

This multiple regression is highly significant, accounting for nearly two-thirds of the variance between experiments (Table IV).

TABLE IV

Analysis of Variance: Multiple Regression of Net Assimilation Rate on Environmental Factors

	<i>n</i>	Sum of squares.	Mean square.	<i>f</i>	<i>p</i>
Regression	5	1651.81	330.36	6.27	<0.01
Remainder	17	899.55	52.91	—	—
Total	22	2551.36			

An attempt has been made to demonstrate separate effects of the five independent variables. The additional variance ascribable to each of the five above that due to the other four is shown in Table V:

TABLE V

Analysis of Variance: Regression of Net Assimilation Rate on Environmental Factors Separately

	<i>n</i>	Sum of squares.	Mean square.	<i>f</i>	<i>p</i>
Regression on <i>L</i> , eliminating <i>D</i> , <i>T</i> , <i>N</i> , <i>S</i>	1	313.27	313.27	5.94	0.01-0.05
Regression on <i>D</i> , eliminating <i>L</i> , <i>T</i> , <i>N</i> , <i>S</i>	1	125.07	125.07	2.37	>0.05
Regression on <i>T</i> , eliminating <i>L</i> , <i>D</i> , <i>N</i> , <i>S</i>	1	2.69	2.69	—	—
Regression on <i>N</i> , eliminating <i>L</i> , <i>D</i> , <i>T</i> , <i>S</i>	1	18.31	18.31	—	—
Regression on <i>S</i> , eliminating <i>L</i> , <i>D</i> , <i>T</i> , <i>N</i>	1	51.35	51.35	—	—
Remainder	17	899.55	52.91	—	—

Thus, light intensity is the only one of the five factors that can be shown from these data to affect the net assimilation rate separately. This is not to say that the other factors have no effect, or that their effects are negligible; the result is a consequence simply of the very close correlation between all five variables. This correlation renders such data as the present ones unsuitable for analysis of the effects of environmental factors on physiological processes; other attempts to use similar data for this purpose have been reviewed on p. 104. In the previous work published by Bolas et al. (1938) a similar analysis was carried out, with only two independent variables—mean light intensity and mean temperature; in their results, the latter but not the former proved to have a significant separate effect on the net assimilation

rate. The discrepancy between this result and that in the present series of experiments is not, however, of importance, for the reasons stated above.

The differences between the mean values for winter and summer of the five environmental factors measured account for 84 per cent. of the difference between the mean net assimilation rates for these periods.

In Table II the rates of dry-weight change (per cent. per hour) of the whole plant in each experiment during each of the four periods into which the day was divided have been given. During the day the plant is consistently (with three exceptions) gaining in dry weight. The mean hourly rates of gain in summer and winter do not differ greatly; the much greater difference in the relative growth-rates over the whole 24 hours must thus be ascribed mainly to the fact that the midsummer day in these latitudes is about twice the length of the midwinter day. The rate of dry-weight increase is rather greater in the morning than in the afternoon, especially in summer; in view of the fact that the mean light intensity in the morning is considerably higher than in the afternoon, this is not surprising. Killian (1933) with *Narcissus* and Melville (1934) in the earlier work with tomato also found the lower light intensity in the afternoon accompanied by a lower assimilation rate.

Between dusk and midnight the plant loses in dry weight. The values as between summer and winter are very discrepant, as also between the earlier and later periods of the night. These differences between summer and winter values are not accounted for by temperature differences, these being very small. Stated as respiration rates the values for summer in the period before midnight would give quite unusually high values. The subsequent gain in weight before dawn in summer commensurate with the gains during the day is also quite unexpected, though a similar observation on cucumbers was made by Henderson & Bolas (private communication), and the difference between the mean rates of change before and after midnight is highly significant. The mean value over the whole night of 0.2 per cent. per hour corresponding with a respiration rate of approximately 3 mg. CO₂ per gm. dry weight on the other hand appears normal. Without further evidence it would appear unwise to speculate on the cause of these differences.

II. Changes in dry weight of organs separately.

In Table VI the percentage gains in dry weight over 24 hours (dawn to dawn) of the various organs are detailed. The mean winter figures range from 2.7 per cent. in the cotyledons to 49.4 per cent. in the seventh leaf, while the range in summer is from 4.0 per cent. to 64.1 per cent., the eighth leaf here showing the greatest gain. The differences between these extremes in summer and in winter are not so great as might be expected from the difference between the corresponding means for the whole plant (8.6 per cent. in winter and 20.8 per cent. in summer). Between the summer and winter rates of gain for the stem, root, and larger leaves (Nos. 2-4), however, the difference is more marked, the summer value being about 3 times that of winter. One may therefore say that in summer it is to these organs, and not

TABLE VI
Change in Dry Weight (per cent.) from Dawn to Dawn

Date of experiment	Root		Stem		Cotyledons		Leaf 1		Leaf 2		Leaf 3		Leaf 4		Leaf 5		Leaf 6		Leaf 7		Leaf 8	
	S.E.		S.E.		S.E.		S.E.		S.E.		S.E.		S.E.		S.E.		S.E.		S.E.		S.E.	
Dec. 7, 1936	- 8.6	7.3	- 15.2	6.4	- 16.3	7.5	- 10.5	4.7	- 10.4	5.5	- 8.8	5.9	- 14.1	6.3	- 4.9	8.4	- 11.1	8.2	+ 17.5	25.0	—	—
Dec. 20, 1936	- 3.6	7.1	- 1.0	7.1	—	—	+ 13.6	4.2	+ 1.5	6.0	+ 19.4	6.5	+ 0.8	6.0	+ 15.8	7.6	+ 28.4	6.0	+ 34.9	9.1	+ 95.4	20.1
Jan. 11, 1937	+ 11.9	7.1	- 12.0	6.0	—	—	+ 13.0	4.4	+ 8.8	5.3	- 13.5	4.7	+ 11.5	5.6	+ 10.5	5.1	+ 28.4	7.0	+ 37.4	10.5	+ 19.7	17.7
Jan. 18, 1937	+ 4.8	7.5	+ 7.3	5.1	—	—	+ 0.6	4.3	+ 2.1	4.7	+ 12.5	4.4	+ 4.8	5.7	+ 9.9	5.1	+ 19.6	4.3	+ 63.3	6.4	+ 28.4	15.1
Feb. 4, 1937	- 7.8	7.1	+ 6.3	4.2	+ 10.0	13.8	+ 8.3	5.0	+ 2.9	6.3	- 0.1	4.8	- 3.3	6.4	+ 14.3	4.8	+ 23.5	4.3	+ 40.6	7.6	- 17.0	22.5
Feb. 15, 1937	- 1.5	7.1	- 7.4	3.9	+ 24.0	12.5	- 0.9	4.2	+ 6.2	5.5	+ 7.2	4.3	+ 7.3	3.7	+ 31.0	5.2	+ 51.0	5.0	+ 54.5	9.0	+ 01.2	15.0
Feb. 23, 1937	- 0.7	7.1	- 15.9	3.7	+ 1.1	4.8	+ 1.6	4.4	+ 4.7	4.7	+ 6.7	3.5	+ 16.4	3.6	+ 24.4	3.7	+ 15.8	6.0	+ 32.4	8.4	- 11.8	24.3
Mar. 8, 1937	+ 3.7	7.3	+ 16.5	4.7	+ 3.2	5.4	+ 13.0	5.7	+ 9.2	5.7	+ 14.6	4.0	+ 25.2	3.7	+ 28.4	3.5	+ 60.0	6.2	+ 82.8	9.2	+ 80.2	14.3
Mar. 23, 1937	+ 9.7	7.0	- 32.6	6.2	+ 11.1	5.6	+ 16.6	4.7	+ 10.9	4.6	+ 13.2	2.8	+ 26.8	3.5	+ 30.9	3.1	+ 16.6	3.8	+ 43.9	4.2	+ 05.5	8.7
Apr. 5, 1937	- 2.1	7.3	- 22.4	6.2	+ 3.4	5.8	+ 6.3	5.3	+ 4.3	4.8	+ 24.4	4.2	+ 24.8	4.1	+ 32.4	3.5	+ 48.9	4.3	+ 61.8	6.4	+ 22.1	11.8
Apr. 12, 1937	+ 11.3	7.0	+ 27.4	5.4	+ 0.8	5.4	+ 4.5	5.1	+ 10.0	5.7	+ 25.7	5.2	+ 31.0	4.1	+ 48.5	2.4	+ 67.5	5.8	+ 82.0	7.3	+ 102.5	10.0
Apr. 28, 1937	- 7.4	7.0	- 18.5	4.3	+ 3.5	5.0	+ 2.8	4.2	- 0.5	4.5	+ 12.8	3.8	+ 10.3	5.0	+ 44.4	4.2	+ 31.0	3.8	+ 42.2	5.1	+ 52.9	8.4
May 6, 1937	+ 9.8	7.1	- 34.4	3.3	- 4.5	5.4	+ 8.1	5.2	+ 21.0	4.0	+ 35.2	3.8	+ 43.0	5.0	+ 57.8	3.0	+ 60.6	4.1	+ 81.2	6.0	+ 100.7	7.8
May 24, 1937	+ 15.8	7.1	- 36.0	5.1	- 7.1	5.8	+ 13.9	6.1	+ 19.8	5.2	+ 28.2	4.7	+ 42.5	3.6	+ 59.0	3.5	+ 95.4	6.6	+ 78.1	5.5	+ 109.7	7.8
June 2, 1937	+ 17.8	7.2	+ 30.2	5.0	+ 0.1	5.3	+ 14.1	5.3	+ 9.4	5.3	+ 11.9	4.0	+ 33.9	4.2	+ 57.0	5.8	+ 91.6	4.8	+ 59.4	8.3	+ 32.6	14.5
July 7, 1937	+ 25.2	7.1	- 33.0	4.4	- 6.7	4.8	+ 15.6	4.7	+ 17.7	5.6	+ 31.8	4.1	+ 41.4	3.6	+ 75.0	4.1	+ 82.5	5.4	+ 85.4	9.6	+ 54.0	10.5
Aug. 17, 1937	- 3.2	7.0	+ 25.7	5.3	+ 3.4	5.3	+ 10.0	6.0	+ 12.7	3.4	+ 21.6	3.0	+ 43.1	4.1	+ 63.0	5.8	+ 79.0	5.8	+ 73.6	6.1	+ 123.9	12.2
Sept. 2, 1937	+ 36.9	7.1	+ 23.3	5.8	+ 0.3	7.9	+ 12.0	5.4	+ 22.6	6.2	+ 27.2	5.3	+ 29.0	4.4	+ 50.5	4.2	+ 79.0	5.8	+ 44.4	10.0	+ 36.7	12.1
Sept. 10, 1937	+ 29.4	7.1	- 23.4	4.5	+ 12.0	6.6	+ 24.4	6.0	+ 37.0	4.5	+ 24.9	5.3	+ 26.9	4.1	+ 42.7	6.3	+ 18.6	5.8	+ 20.3	12.4	+ 25.3	19.4
Sept. 27, 1937	- 7.5	7.1	+ 21.6	6.2	+ 2.8	4.8	+ 2.2	5.3	+ 9.9	4.5	+ 12.4	4.6	+ 23.8	4.6	+ 30.2	3.5	+ 49.0	5.2	+ 64.3	8.6	+ 89.2	13.5
Oct. 11, 1937	+ 26.6	7.3	- 31.2	4.2	+ 12.0	6.7	+ 12.4	8.6	+ 17.7	5.2	+ 10.9	4.5	+ 31.2	3.3	+ 42.1	4.0	+ 33.8	5.6	+ 60.5	9.2	+ 84.7	19.3
Nov. 11, 1937	+ 7.9	7.1	- 15.8	4.2	- 15.3	4.2	+ 14.1	6.1	+ 5.7	4.8	+ 13.9	4.2	+ 17.9	3.6	+ 28.0	4.7	+ 25.2	4.7	+ 37.0	8.4	+ 33.5	12.1
Nov. 25, 1937	- 1.5	7.0	+ 11.0	4.5	—	—	- 5.6	7.9	+ 5.3	4.9	+ 1.8	5.2	+ 2.5	4.0	+ 10.2	3.6	+ 17.1	5.2	+ 40.2	11.4	+ 10.8	18.8
Dec. 7, 1937	- 8.2	7.1	- 2.6	4.7	—	—	+ 8.5	3.8	+ 9.0	3.5	+ 9.3	4.6	+ 9.9	4.2	+ 11.0	4.5	+ 19.3	7.1	+ 61.5	15.2	—	—
Mean (winter)	+ 4.3	2.0	+ 9.9	1.4	+ 2.7	2.9	+ 6.1	1.5	+ 4.8	1.4	+ 7.3	1.3	+ 11.0	1.3	+ 19.8	1.4	+ 27.7	1.7	+ 49.4	3.2	+ 43.1	5.4
Mean (summer)	+ 13.4	2.1	+ 27.9	1.5	+ 4.0	1.7	+ 11.7	1.6	+ 15.0	1.5	+ 23.4	1.3	+ 32.6	1.2	+ 49.7	1.3	+ 63.1	1.6	+ 61.9	2.3	+ 64.1	3.7
Mean (all expts.)	+ 8.5	1.5	+ 18.1	1.0	+ 3.4	1.6	+ 8.7	1.1	+ 9.5	1.0	+ 14.7	0.9	+ 20.9	0.9	+ 33.5	1.0	+ 43.9	1.1	+ 55.1	2.0	+ 53.6	3.3

to the oldest or the youngest leaves, that most of the additional assimilate formed goes.

In Fig. 3 these changes are expressed as percentages of the amounts necessary to maintain the initial proportions of total dry weight among the organs of the plant. It will be observed that neither in winter nor in summer does the root receive sufficient to retain this proportion, while the stem is receiving more; in consequence at this stage the root constitutes a decreasing and the stem an increasing proportion of the plant's dry weight. As might

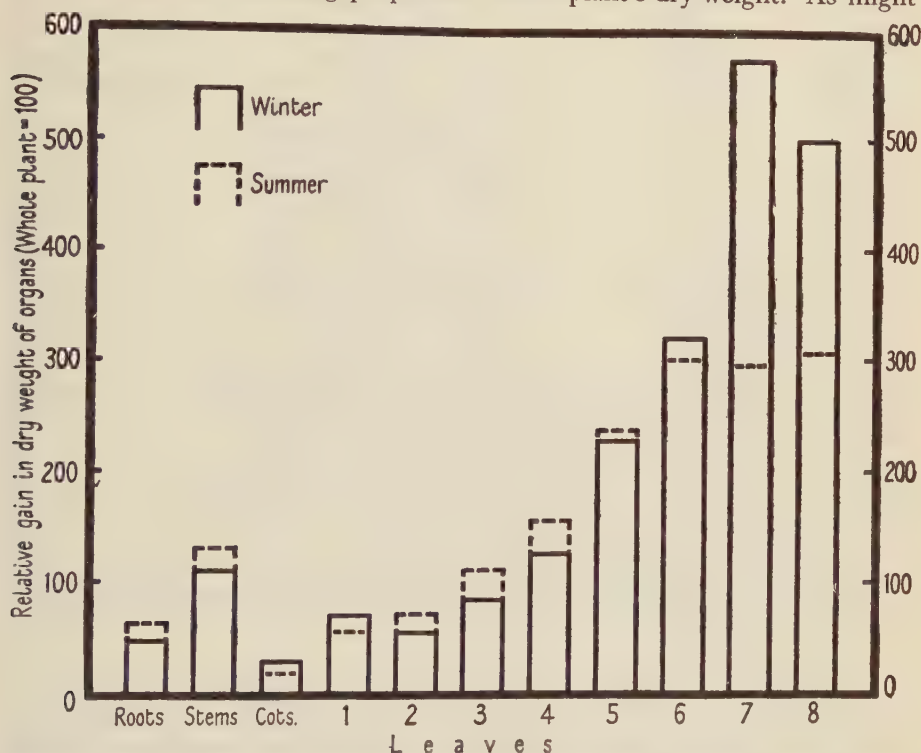


FIG. 3. Relative increase in dry weight of the various organs of the plant during a period of 24 hours, expressed as a percentage of the relative increase of the whole plant.

be expected, the older leaves accumulate less than their initial weight would demand, while the young leaves receive considerably more; the difference between summer and winter is striking, for whereas in winter the rate of increase in dry weight of the young leaves is 5 or 6 times that of the plant as a whole, in summer it is barely 3 times. Moreover, in summer the sixth, seventh, and eighth leaves all behave alike, while in winter the sixth leaf is considerably below the other two.

Fig. 4 presents a picture of the distribution of the assimilate at the end of the 24-hour period—that is, the percentage of the total increment in dry weight then to be ascribed to the various organs. The absolute amount going to any organ is related to its initial weight, so that the percentage of

the total increment passing to the young rapidly developing leaves is quite small.

Table VII shows the mean changes in dry weight during the four periods of the 24 hours which lead to these final results.¹ The root shows in summer the increase taking place mainly during the daytime; during the night there

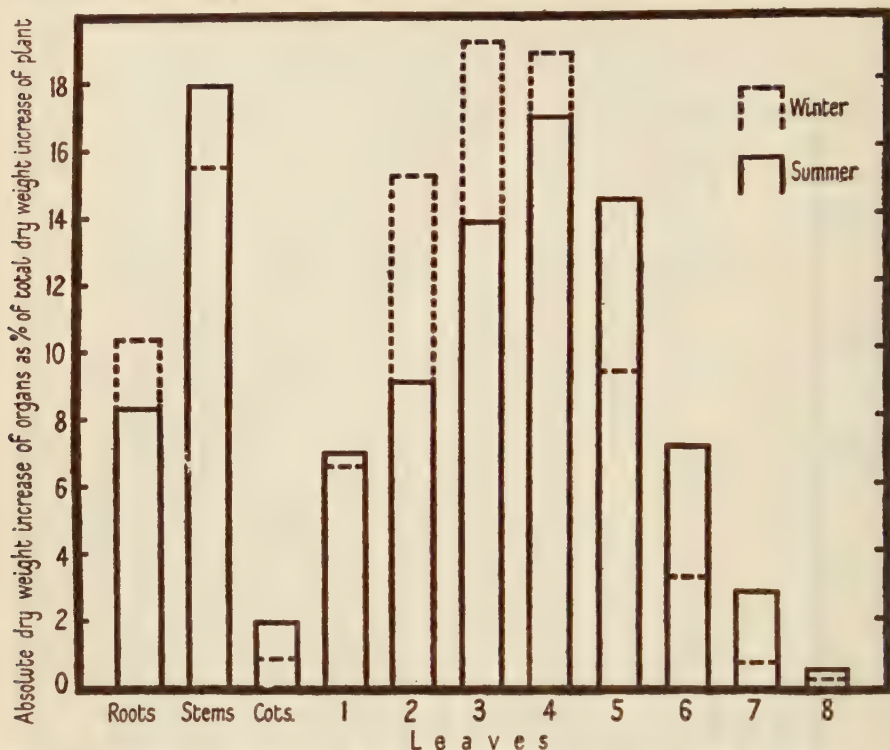


FIG. 4. Increase in dry weight of the various organs of the plant, during a period of 24 hours, expressed as a percentage of the total absolute increase of the whole plant.

is a net loss. During the winter its dry weight remains virtually unchanged at the end of the short day, but increases during the evening. This means that in winter translocation is slight during the day-time, while in the longer summer day translocation of the material formed during the day is almost completed before nightfall, and suggests that there is a lag between the peak of the assimilation curve and the time of most active translocation, as found by Dijkstra (1937).

In the case of the stem, the main increase in dry weight occurs during the day-time both in summer and in winter, and the net rate of gain during the night is slow; the rate of gain in the afternoon is just significantly greater than

¹ This summary table is based on eleven tables giving in detail the change in dry weight of each organ (root, stem, cotyledon, and leaves 1-8) for each of the 26 experiments. As these tables with their standard errors contain over 2,200 entries they have had to be omitted. They are available in the original Ph.D. thesis which can be consulted at the University of London.

TABLE VII

Change in Dry Weight (per cent. per hour) of the Various Organs of the Tomato Plant throughout the Twenty-four Hours. Mean Values are given for Winter and Summer, and for the Year as a whole

	Dawn-midday.		Midday-dusk.		Dusk-midnight.		Midnight-dawn.	
	S.E.		S.E.		S.E.		S.E.	
Root								
Winter . .	—0·03	0·38	—0·03	0·37	0·78	0·29	—0·26	0·29
Summer . .	1·20	0·26	1·01	0·26	—0·82	0·57	0·17	0·54
General mean .	0·56	0·23	0·46	0·23	0·08	0·31	—0·08	0·29
Stem								
Winter . .	0·47	0·28	1·04	0·27	0·35	0·20	—0·01	0·21
Summer . .	1·09	0·20	1·86	0·20	—0·29	0·39	1·20	0·36
General mean .	0·77	0·18	1·42	0·17	0·06	0·21	0·52	0·20
Cotyledons								
Winter . .	0·96	0·52	—0·38	0·46	—0·43	0·38	0·32	0·41
Summer . .	0·34	0·22	0·42	0·21	—1·27	0·46	0·76	0·42
General mean .	0·56	0·25	—0·01	0·24	—0·86	0·30	0·56	0·29
Leaf 1								
Winter . .	1·23	0·24	0·52	0·24	—0·05	0·18	—0·22	0·19
Summer . .	1·28	0·20	0·43	0·20	—1·21	0·43	0·83	0·41
General mean .	1·25	0·15	0·48	0·15	—0·56	0·21	0·24	0·21
Leaf 2								
Winter . .	1·09	0·28	0·48	0·26	—0·44	0·20	—0·14	0·21
Summer . .	1·36	0·18	0·86	0·19	—1·24	0·39	0·09	0·38
General mean .	1·22	0·17	0·66	0·17	—0·79	0·20	—0·04	0·21
Leaf 3								
Winter . .	1·26	0·26	1·54	0·25	—0·71	0·19	—0·38	0·19
Summer . .	2·01	0·16	1·18	0·16	—1·32	0·34	0·65	0·32
General mean .	1·62	0·16	1·37	0·16	—0·98	0·19	0·07	0·18
Leaf 4								
Winter . .	1·54	0·27	0·97	0·25	—0·31	0·19	—0·38	0·19
Summer . .	2·35	0·16	1·49	0·16	—0·95	0·32	0·62	0·30
General mean .	1·93	0·16	1·21	0·15	—0·59	0·18	0·06	0·17
Leaf 5								
Winter . .	1·23	0·28	2·11	0·27	0·12	0·19	—0·13	0·19
Summer . .	2·42	0·16	2·39	0·16	—0·36	0·36	1·01	0·34
General mean .	1·80	0·16	2·24	0·16	—0·09	0·19	0·37	0·18
Leaf 6								
Winter . .	1·46	0·33	2·26	0·31	0·34	0·23	0·62	0·23
Summer . .	2·61	0·19	2·42	0·19	0·09	0·42	2·07	0·40
General mean .	2·01	0·19	2·33	0·19	0·23	0·22	1·26	0·22
Leaf 7								
Winter . .	3·18	0·54	3·24	0·53	0·67	0·36	1·18	0·36
Summer . .	2·93	0·29	2·91	0·29	—0·87	0·60	0·88	0·57
General mean .	3·05	0·30	3·09	0·30	—0·04	0·34	1·04	0·34
Leaf 8								
Winter . .	0·68	1·05	2·52	0·99	1·97	0·72	0·71	0·69
Summer . .	2·56	0·34	3·58	0·46	—2·89	0·98	0·98	0·89
General mean .	1·66	0·53	3·06	0·54	—0·35	0·60	0·84	0·56

that before midday. The fact of the rate of assimilation of the whole plant being greater before than after midday supports the view that there is a lag of translocation behind assimilation. That most of the translocation to the stem and root occurs during the day-time accords well with the results of Bolas et al. (1938), who found that during a 7-hour assimilation period 72 per cent. of the expected eventual translocation took place.

The older leaves in general gain in dry weight throughout the day-time, though indeed more rapidly during the morning than the afternoon; the difference between these two periods is greater than in the plant as a whole. This result is at variance with that of Porter (1937), who found that tomato leaves increased in dry weight more in the afternoon than in the morning; on the other hand, these figures do not show the afternoon decrease in dry weight that has often been reported for mature leaves (see p. 103); perhaps this is because most of the leaves are not yet fully mature. In the cotyledons and the first and second foliage leaves the rate of gain during the day-time is less than that of the plant as a whole, either because their assimilation rate is low or because the rate of translocation from them is high. This statement does not hold for the short winter morning, which suggests that the second explanation is the true one. From the third leaf upwards the rates of increase during the day-time always exceed those of the plant as a whole, with the single exception of the eighth leaf during the winter morning. This suggests that this very small leaf is largely dependent upon external supplies, and that in the short winter morning translocation to it has not yet begun. The most rapid increase in dry weight during the day-time is to be found in the seventh leaf, except in the summer afternoon, when it yields place to the eighth; Melville (1934) also found that the smallest leaf but one was most active. The rates of increase in dry weight found here for the older leaves are quite similar to those recorded in the literature. Sachs' (1884) figures, for instance, range from 1.22 per cent. per hour in *Cucurbita* to 2.09 per cent. per hour in *Helianthus*, and Watson (1936) found for potato between 6.00 and 9.00 a.m. an increase of 1 per cent. per hour.

During the night the older leaves, up to and including the third, are losing weight more rapidly than the plant as a whole. This is also true of the fourth leaf in winter but not in summer, showing that in winter more leaves contribute to the support of the younger and non-assimilating parts of the plant, which is doubtless connected with their earlier senescence. The younger leaves in most cases lose less than the whole plant, and often gain while the rest of the plant is losing in dry weight. In the winter evening, for instance, the eighth leaf is gaining at the rapid rate of 2.77 per cent. per hour, while the whole plant is slightly decreasing in dry weight. In summer, surprisingly, the eighth leaf is losing rapidly in weight during the evening—nearly 3 per cent. per hour on average—and the seventh leaf is also losing, though less rapidly. These facts confirm the impression that whereas in winter translocation continues when the short day has come to a close, in summer it almost ceases at dusk. The mean rates of loss in dry weight of the older leaves during the

night recorded here, about 0.4 per cent. per hour, are rather low when compared with other figures in the literature. Sachs (1884) found *Helianthus* leaves lost 1.2 per cent. per hour and *Cucurbita* leaves 1.4 per cent. per hour. Denny (1933) with peanuts obtained a value of 0.4 per cent. per hour, but with a bean variety 1.1 per cent. per hour, while Watson's (1936) figure for potato was 1.7 per cent. per hour.

SUMMARY

1. The dry-weight changes in the various organs of the tomato plant at the eight-leaf stage were followed in experiments continued through the whole 24 hours and performed at all times of the year.

2. The regression of the dry weights of the various organs on the lengths of the leaves and of the stem was used to follow the changes in these organs without mutilation of the plant during life.

3. For determining the dry weight a special drying-oven was constructed in which the material is placed in tubes surrounded by hot water; special spring balances were also employed.

4. The plants for the experiments were selected for uniformity in size. Only plants with alternate leaves were used, those in which the first two leaves were opposite being rejected.

5. From the dry-weight increases of the whole plant the relative growth-rates on the days of the various experiments were calculated. During the summer months they were rather higher than most of those recorded for other plants. This is the result of the high proportion that the leaves form of the total plant material; the mean net assimilation rate in the experiments during the six months of summer is slightly below the average for other plants.

6. A multiple regression of net assimilation rate on light duration, light intensity, day and night temperature, and humidity is calculated and shown to be highly significant; only light intensity, however, can be shown to have a *separate* effect on the net assimilation rate.

7. The hourly rate of increase in dry weight of the whole plant during the day does not differ very greatly in summer and winter; the much greater difference in relative growth-rate can largely be ascribed to the difference in duration of the light period.

8. The rate of increase in dry weight of the whole plant is higher in the morning than in the afternoon; this is probably due to the difference in light intensity.

9. During the evening the plant loses in dry weight much more rapidly in summer than in winter, although the mean temperatures differ very little.

10. The additional amount of assimilate produced in summer over that produced in winter goes mainly to the stem, the root, and the largest leaves (the second, third, and fourth), while the rates of increase of the older and the younger leaves do not differ greatly as between summer and winter.

11. At this stage the proportion of the plant formed by the root is decreasing, while that formed by the stem is increasing.

12. In proportion to the increase of the whole plant, the gain in dry weight of the young leaves is much greater in winter than in summer.

13. From the distribution of the dry-weight changes of the organs through the 24 hours, it is deduced that most translocation takes place during the day-time in summer, and that the time of greatest translocation is somewhat later than the time of maximum assimilation.

APPENDIX: STATISTICAL ASPECTS OF THE INVESTIGATION

I. The measurements used as bases for estimation of changes in dry weight

(a) *Leaves.* The measurement of the leaf that was most likely to be closely related to its dry weight was its area. In view of the impracticability of measuring the area of every leaf in samples of the size contemplated this relationship was not investigated. However, an approximation to it was made by measuring the length and maximum breadth of each pinna of the leaf, and relating the dry weight to the sum of the products of these two measurements. The ratio of the leaf dry weight to this quantity had a coefficient of variability ranging from about 10 per cent. in the largest to 30 per cent. in the smallest leaves.

These measurements, however, were rather laborious, there being on some of the larger leaves as many as 10 pinnae; the method could therefore hardly have been used for a large batch of plants. But since in a given batch of plants the shape of leaves of the same age differs only slightly, it was possible that a single measurement of the leaf would give a sufficiently close approximation. Darrow (1932) found that the area of the strawberry leaf was related to the length of the terminal leaflet with an error of 5 per cent.

When the lengths of tomato leaves from the axil to the tip were plotted against their fresh and dry weights (Fig. 5) the points were found to lie close to a curve which was little different from that of the function $w = al^2$, where w is the weight and l the length, a being a constant. Accordingly, the coefficients of variability of the ratio weight/length² were calculated for the leaves of different ages, and are given in Table VIII.

TABLE VIII
Coefficients of Variability of Observations on Leaves

		Fresh weight	Fresh weight		Dry weight
			Length ²		Dry weight
					Length ²
Leaf 1	.	19.9	9.5	21.7	10.7
Leaf 2	.	17.9	13.5	24.7	15.7
Leaf 3	.	33.3	13.3	34.8	13.2
Leaf 4	.	38.0	11.5	41.8	12.9
Leaf 5	.	54.4	12.5	62.0	14.6
Leaf 6	.	70.9	10.9	75.2	19.0
Leaf 7	.	78.0	7.7	94.5	24.8
Leaf 8	.	118.5	9.3	114.9	20.3
Leaf 9	.	84.8	23.6	107.4	42.0

It is seen that the length of the leaf is very little inferior to the sum of the

products of pinna length and breadth as an indication of the weight of the leaf. The functions $\log w = a \log l$ and $w = al + bl^2$ showed a slightly closer relation than $w = al^2$. Since the first expression was easier to apply, and also lent itself to the direct calculation of relative changes, it was decided to use this function.

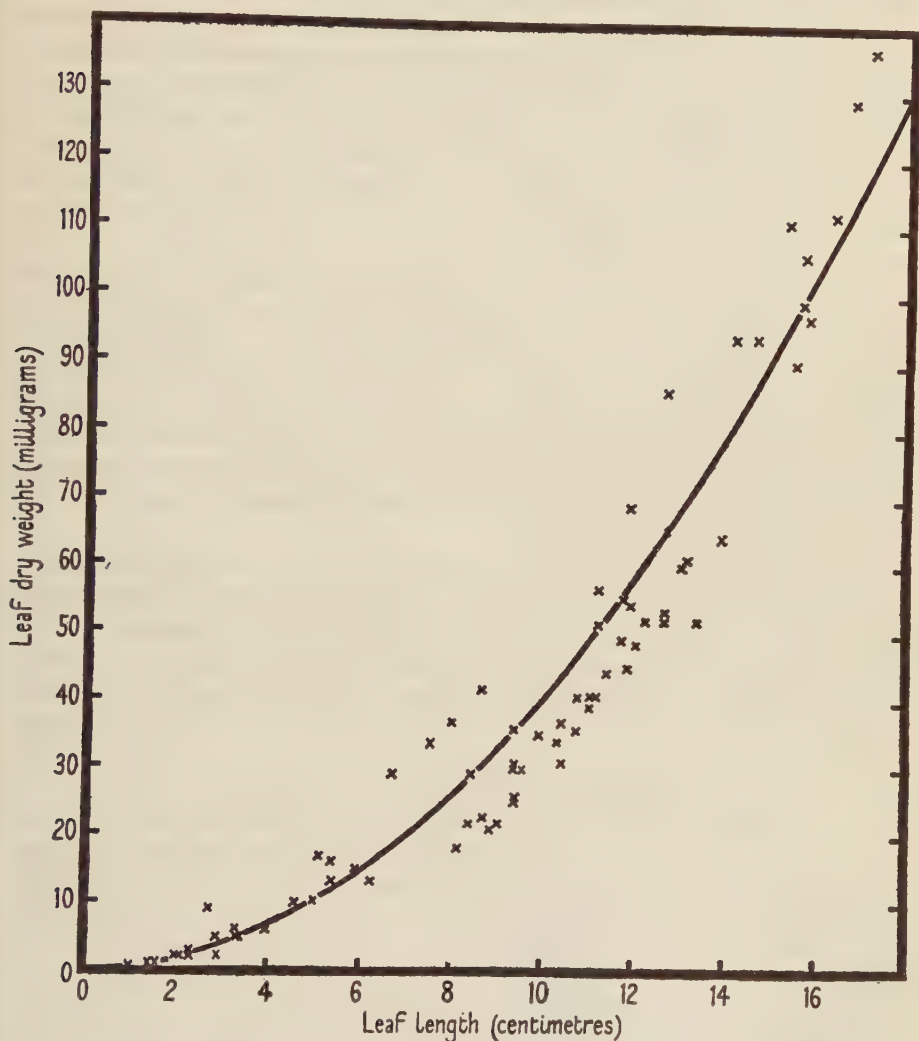


FIG. 5. Correlation between leaf length and dry weight, using all the leaves of a batch of plants irrespective of age. The curve drawn is that of closest fit of the type $y = ax^2$.

In the observations so far recorded the leaf length had been measured from the axil, and the leaf had been separated from the stem at that point. It was possible, however, that to measure from, or to cut off at, the basal leaflet, as was done by Porter (1937), might give better results. The two measurements were compared in two experiments, and it appeared that the

most satisfactory results were likely to be obtained by measuring the leaf from the axil, and separating it at that point also.

(b) *Stems*. In the case of the stems, the following measurements were investigated as a possible basis for estimation of the dry weight: length (from cotyledons to base of terminal bud), diameter at cotyledons, and diameter just below terminal bud, the latter two measurements being made by means of vernier callipers. Various functions of these measurements were calculated, and of these $\text{length} \times (\text{basal diameter})^2$ seemed to give the most satisfactory estimate of the dry weight. Its advantage over the square of the length, however, was not striking. Moreover, the calliper measurement took considerably more time than any ruler measurement, and involved the risk of damaging the plant. Accordingly the length only was measured, and a logarithmic relation was used as in the case of the leaves.

(c) *Cotyledons*. It was not at first thought that the length of the cotyledons would bear any close relation to their dry weight, since they vary little in length but differ considerably in thickness and fleshiness, features difficult to measure. Accordingly some relation of the cotyledon dry weight to the size of the rest of the plant was sought to enable the former to be estimated by measurement. Correlation coefficients with the lengths of three representative leaves and with that of the stem were calculated for twelve batches of plants. Of the measurements used the length of the first leaf was most closely related to the dry weight of the cotyledons.

In later work the lengths of the cotyledons themselves were also measured with the surprising result, in view of the considerations above, that the length of the cotyledons was a more satisfactory means of estimating their dry weight than the length of the first leaf. In Table IX the coefficients of variability of these two ratios and of the cotyledon dry weight are given for six batches of plants:

TABLE IX
Coefficients of Variability of Observations on the Cotyledons

Month.	Cotyledon dry wt.	Cotyledon dry wt. Leaf \times length ² .	Cotyledon dry wt. Cotyledon length ² .
June . . .	6.3	6.2	10.3
July . . .	17.1	16.4	11.3
August . . .	19.8	16.8	7.8
August . . .	30.0	23.5	17.6
September . . .	16.3	13.6	11.8
September . . .	15.6	26.5	19.5

It will be seen that in the case of the cotyledons the improvement in estimation obtained by preliminary measurement is smaller than in the case of the foliage leaves. It is unfortunate that the desirability of measuring the cotyledon length was not discovered until the series of experiments had been half completed. However, it was thought worth while to use this measurement in the later experiments, which consequently have smaller errors than the earlier ones in the changes in cotyledon dry weight.

(d) *Roots.* For estimating the dry weight of the roots no direct measurement was possible except the width of the hypocotyl which involved the risk of injury; it was necessary, therefore, to rely on the relation between the size of the roots and of the aerial parts of the plant. It was found that the highest correlations were with the length of leaf 5. In Table X the coefficients of variability of the ratio of root dry weight to the length of that leaf are compared with those of the former quantity itself for nine batches of plants.

TABLE X. *Coefficients of Variability of Observations on the Root*

Month.	Root dry wt.	Root dry wt.	
		Leaf 5 length	
February	12.8	13.4	
March	11.8	8.3	
April	19.7	12.3	
May	14.3	10.0	
June	17.2	13.9	
August	26.8	11.3	
October	19.8	8.1	
November	27.3	16.9	
December	27.1	19.1	

From this table it appears likely that the estimation of the root dry weight on the basis of the length of the fifth leaf will reduce the variance by about 50 per cent. This method of estimating dry weight was used in a logarithmic form, as in the other organs.

(e) *The whole plant.* A similar method to those used for estimating the dry weights of the various organs could obviously be devised for estimating the dry weight of the whole plant. This could be done most accurately by a multiple regression of the plant dry weight on all the measurements made, which, however, would be an extremely laborious calculation, and could probably be replaced without much loss by a regression on one or a few measurements only. It was found in fact that by calculating a multiple regression of plant dry weight on the lengths of the second and seventh leaves it was possible to reduce the variance by about 70 per cent. In the present work, however, a better estimate of the dry-weight change of the whole plant could be derived from the dry-weight changes found for the various organs. These changes were found as logarithms—i.e. they were relative—and it was necessary to put them on an absolute basis. This was done by finding the mean of the logarithms of the initial dry-weight values for each organ, and then finding what change in absolute dry weight the mean logarithmic change represented on this mean initial dry weight. The sum of these absolute changes in the various organs was taken as the change in the dry weight of the whole plant. An accurate estimate of the error of this change was difficult to obtain since it depended upon the correlation between the deviations of the dry weights of the various organs of the same plant from their respective weight/length regressions. These deviations proved to be significantly correlated, so it would not have been legitimate to use as the standard error of the change in plant dry weight the square root of the sum of the

variances for the various organs. To calculate the true standard error of each change, all the fifty-five correlation coefficients between the deviations would be required. Accordingly, it was decided to quote as a maximal value for the error that derived from the multiple regression on the lengths of the second and seventh leaves. This is the figure given in the column headed 'S.E.' in all tables listing changes in the dry weight of the whole plant.

In Table XI the rates of change (per cent. per hour) found by this method in two experiments are compared with those found using the regression of plant dry weight on the lengths of the second and seventh leaves, and also by direct sampling without the use of measurements:

TABLE XI
*Rates of Change in Dry Weight of Whole Plant (per cent. per hour)
Calculated by Three Methods*

	By sampling.		By regression on lengths of leaves 2 and 7.		By sum of changes in various organs	
		S.E.		S.E.		S.E. (max.).
<i>June 2, 1937.</i>						
Dawn-midday	+1.79	0.76	+1.75	0.40	+2.02	0.40
Midday-dusk	+0.50	0.79	+0.42	0.40	+0.30	0.40
Dusk-midnight	-1.12	2.25	-1.12	1.14	-0.94	1.14
Midnight-dawn	-0.20	2.12	+0.10	1.11	+0.36	1.11
<i>Dec. 7, 1937.</i>						
Dawn-midday	+1.69	1.65	+1.63	0.94	+1.00	0.94
Midday-dusk	-0.99	1.68	-0.79	0.94	-0.20	0.94
Dusk-midnight	+0.67	0.85	+0.42	0.56	+0.24	0.56
Midnight-dawn	-2.20	0.88	-1.66	0.54	-2.11	0.54

II. Estimation by measurement compared with estimation by pairing

The use of measurements to estimate the initial dry weight of the organs of the experimental plants represented an improvement on the pairing method, and in the case of the smaller leaves a very substantial one. In Table XII are given the results of two experiments to test this point:

TABLE XII
*Root Mean Square Deviations (per cent.) of Estimated from Actual
Values for Dry Weight*

	Experiment A.		Experiment B.	
	By measurement.	By pairing.	By measurement.	By pairing.
Cotyledons	7.7	6.1	—	—
Leaf 1	9.1	11.7	11.3	13.8
Leaf 2	8.7	12.3	12.5	29.0
Leaf 3	9.4	10.0	9.1	16.5
Leaf 4	10.2	7.0	11.2	9.3
Leaf 5	6.6	6.9	9.5	18.6
Leaf 6	7.7	9.9	14.0	30.5
Leaf 7	6.2	17.9	10.8	36.7
Leaf 8	13.0	23.5	20.0	44.3
Stem	4.8	3.2	17.2	16.6
Root	11.6	12.5	14.8	11.9

In the experiments recorded in Table XII the two batches of plants had been sampled simultaneously. A further experiment was carried out to show the comparative efficiency of the two methods in determining changes in dry weight. The results of this experiment, which took place between 9 a.m. and 5 p.m. on a spring day, are given in Table XIII. The percentage changes given by direct sampling, without any attempt to estimate the initial dry weight of the final sample, are included, as well as the estimates of change by the pairing method and by use of the measurements.

TABLE XIII

Changes in Dry Weight (per cent.) Estimated by Three Methods

	By sampling.		By pairing.		By measurement.	
		S.E.		S.E.		S.E.
Cotyledons . . .	+2.6	19.7	+5.0	16.1	-0.5	12.5
Leaf 1 . . .	+6.3	14.6	+10.8	8.6	+3.2	5.2
Leaf 2 . . .	-3.2	11.8	+4.1	16.4	+1.0	5.6
Leaf 3 . . .	-4.2	10.5	-2.9	8.1	-2.4	4.9
Leaf 4 . . .	+8.0	12.7	+8.8	4.8	+9.0	5.1
Leaf 5 . . .	-3.8	16.5	-3.6	11.6	+2.0	4.4
Leaf 6 . . .	+12.8	25.6	+14.2	19.7	+14.0	5.8
Leaf 7 . . .	+39.5	48.8	+77.8	85.5	+32.0	10.7
Leaf 8 . . .	+47.7	50.3	+78.5	72.0	+2.2	5.0
Stem . . .	+5.5	15.7	+4.9	7.8	+6.3	3.3
Root . . .	+6.3	11.2	+10.6	12.5	+17.0	11.4

From these tables it will be seen that the method of estimating dry weight by measurement not only makes more plants available for sampling than the pairing method, but also substantially increases the precision of the results.

III. Calculation of the dry-weight changes

The first step in the computation of the results was the conversion of the dry weights and measurements of the organs into logarithmic values. All subsequent operations were carried out with the logarithms and not with the absolute or percentage values. Conversion of the logarithms into percentage changes was only carried out as a last stage in the calculations.

It was first necessary to know to what extent common estimates of the regression coefficients for the different organs, samples and experiments, and of the errors, could be used.

(i) Variation in regression coefficients.

(a) *Between samples.* In any organ it was possible that the regression of log dry weight on log initial length might vary between the batches sampled at different times of day. This was tested as follows: The regression coefficients were calculated separately for each of the nine samples in an experiment, from the expression:

$$b = \frac{Sxy}{Sx^2},$$

where x represents the deviation of the log length from the mean for that sample, y the deviation of log dry weight, and S indicates summation. A

regression coefficient was also calculated for the whole experiment, this being the sum of the values for Sxy for the different samples divided by the sum of the different values of Sx^2 .

The residual sums of squares were then calculated by the expression

$$S(y-Y)^2 = Sy^2 - bSxy$$

in both cases—using the single regression coefficient for the whole experiment and the nine separate regression coefficients for the separate samples; the difference between these two values of the residual sum of squares represented that between the separate regression coefficients. The variance from this source could then be compared by the f test with the error variance when the separate regressions had been eliminated.

This process was carried out on the figures for stems, roots, cotyledons, and three representative leaves, in four experiments chosen at random. The result is shown in Table XIV.

TABLE XIV
The Variance between Regression Coefficients for Different Samples in the Same Experiment

	<i>n</i>	Mean square.	Error <i>n</i> .	Error mean square.	<i>f</i>	<i>p</i>
Cotyledons (a)	16	0.0034	77	0.0037	—	>0.05
Cotyledons (b)	15	0.0025	101	0.0016	1.55	>0.05
Root . . .	32	0.0039	210	0.0042	—	>0.05
Stem . . .	32	0.0026	210	0.0019	1.38	>0.05
Leaf 1 . . .	31	0.0018	199	0.0018	1.01	>0.05
Leaf 4 . . .	31	0.0013	199	0.0013	1.01	>0.05
Leaf 6 . . .	32	0.0012	197	0.0019	—	>0.05

(a) Regression on length of leaf 1. (b) Regression on length of cotyledons.

It will be seen that there is every reason to suppose that a common estimate of the regression coefficient may be used for all the samples in a given experiment. Since all the three leaves represented in Table XIV agreed in this respect, it was not considered necessary to carry out similar calculations for the other five leaves.

(b) *Between experiments.* The calculations, similar to those described in the preceding section, were performed to find whether the regression coefficients derived from the various experiments were significantly different from one another. The results, for the same organs as before, are given in Table XV.

TABLE XV
The Variance between Regression Coefficients in Different Experiments

	<i>n</i>	Mean square.	Error <i>n</i> .	Error mean square.	<i>f</i>	<i>p</i>
Cotyledons (a)	12	0.0044	620	0.0038	1.16	>0.05
Cotyledons (b)	10	0.0124	553	0.0025	5.01	<0.01
Root . . .	25	0.0117	1545	0.0037	3.15	<0.01
Stem . . .	25	0.0063	1542	0.0019	3.34	<0.01
Leaf 1 . . .	25	0.0159	1491	0.0018	8.94	<0.01
Leaf 4 . . .	25	0.0043	1464	0.0015	2.90	<0.01
Leaf 6 . . .	25	0.0124	1480	0.0022	5.60	<0.01

(a) Regression on length of leaf 1. (b) Regression on length of cotyledons.

In all organs, except the cotyledons in those early experiments where their length was not measured, the regression differs significantly from experiment to experiment. Therefore separate regression coefficients were computed and used for the different experiments.

(c) *Between organs.* A further question was whether a common regression coefficient could be used for the leaves of different ages in a given experiment. Therefore in five experiments chosen at random the calculations similar to those already described were carried out. The results are given in Table XVI:

TABLE XVI
*The Variance between Regression Coefficients for Leaves
of Different Ages*

Date of experiment.	<i>n</i>	Mean square.	Error <i>n</i> .	Error mean square.	<i>f</i>	<i>p</i>
Jan. 18, 1937 . . .	7	0.00175	467	0.00295	1.68	>0.05
Feb. 4, 1937 . . .	7	0.00839	466	0.00429	1.95	0.05
May 24, 1937 . . .	7	0.01092	412	0.00221	4.95	<0.01
Sept. 10, 1937 . . .	7	0.02077	469	0.00502	4.14	<0.01
Nov. 11, 1937 . . .	7	0.00356	474	0.00281	1.27	>0.05

In the five experiments listed, the regression coefficients for the leaves of different ages differ significantly in two cases, and in one the differences border on significance. It was therefore considered advisable to use separate regression coefficients.

(ii) *Variation in error estimates.*

Even though the regression coefficients varied between the different organs and experiments, it was quite possible that the variance of the dry-weight values when this regression had been eliminated (that is, the variance of the dry weight for a given length) would not be significantly different in the various organs and experiments, and that accordingly a common estimate of error could be used (cf. Hendricks, 1937). This possibility was therefore tested.

(a) *Between experiments.* The criterion for testing the agreement of a set of variances among themselves is L_1 , for which tables have been compiled by Nayer (1936). From the variances in the different experiments for the same six organs given in Table XV values for L_1 have been computed, and these are listed in Table XVII below:

TABLE XVII
Agreement between Variance Estimates in Different Experiments

	L_1	<i>f</i>	<i>k</i>	<i>p</i>
Cotyledons (a) . . .	0.845	51	12	<0.01
Cotyledons (b) . . .	0.921	50	11	<0.01
Root	0.965	59	26	<0.01
Stem	0.929	59	26	<0.01
Leaf 1	0.964	57	26	<0.01
Leaf 4	0.912	56	26	<0.01
Leaf 6	0.914	57	26	<0.01

(a) Regression on length of leaf 1. (b) Regression on length of cotyledons.

Thus in every case it is unjustifiable to use a common estimate of error for the various experiments, for if a pooled estimate is used it will lead to over-estimation of the significance of differences (Yates and Cochran, 1938).

(b) *Between organs.* As might be expected, the error variances of the log dry weights of the various organs after elimination of the regression on log length were very different. The lumped figures for all the experiments are given in Table XVIII.

TABLE XVIII
Error Variances for Different Organs

	Mean error variance.	Degrees of freedom.
Cotyledons (a)	0.003808	632
Cotyledons (b)	0.002641	563
Root	0.003703	1545
Stern	0.001873	1542
Leaf 1	0.001781	1491
Leaf 2	0.001859	1487
Leaf 3	0.001525	1496
Leaf 4	0.001497	1464
Leaf 5	0.001613	1468
Leaf 6	0.002212	1480
Leaf 7	0.005322	1453
Leaf 8	0.013250	1208

(a) Regression on length of leaf 1. (b) Regression on length of cotyledons.

Among these figures it seemed that the variances for the five older leaves were rather similar, so their agreement was tested separately, for each of the five experiments included in Table XVI. The values of L_1 are given in Table XIX below:

TABLE XIX
Agreement between Variance Estimates for Leaves 1-5

Date of experiment.	L_1	f	h	p
Jan. 18, 1937	0.980	59	5	>0.05
Feb. 4, 1937	0.958	61	5	<0.05
May 24, 1937	0.902	50	5	<0.01
Sept. 10, 1937	0.948	60	5	<0.01
Nov. 11, 1937	0.959	61	5	<0.05

In all but one of the five experiments the variance estimates for these five leaves differed significantly. Therefore separate estimates for the leaves of different ages were used throughout.

Incidentally it is interesting to note that, in both error variances and regression coefficients, the differences among the older leaves were much less marked in the winter months than in the summer.

(iii) *Method of calculation of dry-weight changes.*

The regression coefficients and the error variances having been calculated it was possible to proceed to the estimation of the dry-weight changes and

their errors. The mean change in the log dry weight over one period in any experiment was given by:

$$\bar{y}_2 - \bar{y}_1 - b(\bar{x}_2 - \bar{x}_1)$$

where \bar{y}_1 and \bar{x}_1 are the mean log dry weight and length of the initial sample, \bar{y}_2 and \bar{x}_2 are the mean log dry weight and length of the final sample, and b is the regression coefficient.

For the variance of the change the following expression (see Bliss, 1935) was used:

$$\frac{S^2}{n_1} + \frac{S^2}{n_2} + s^2(\bar{x}_2 - \bar{x}_1)^2,$$

where S^2 is the error variance of the log dry weight when the regression has been eliminated, s^2 is the variance of the regression coefficient, and n_1 and n_2 are the numbers of organs in the two samples.

In order to obtain the rates of change per hour the mean logarithmic change found as described above was divided by the length of the experimental period. The quotient was converted to a percentage change per hour; it is these figures that are used in Tables II and VII. The standard error of the mean logarithmic change was divided by the number of hours, and the resulting standard error of the rate of logarithmic change was converted into a percentage.

Since the standard errors of the rates of change differ considerably from experiment to experiment, the desirability of using a weighted mean had to be considered. There was, however, an inverse relation between the rate of change and its standard error, and according to Yates and Cochran (1938) an unweighted mean is preferable in such a case. This relation arises from the greatest rates of change being in the summer, when the long day and the short night render the precision of the diurnal rates of change high and that of the nocturnal rates of change low.

It had originally been hoped that this series of experiments would lend themselves to an analysis of variance, but the data proved to depart altogether too much from orthogonality for this to be practicable. In the first place, a number of samples were entirely missing; in addition in other samples a number of individual results were lacking because the leaf had been lost during manipulation, which not infrequently happened in the case of the smallest leaves. The magnitude of the losses from these and other causes may be judged from the fact that if all the samples had been complete, the degrees of freedom for each organ in Table XVIII would have been 1569. A second cause of non-orthogonality was the demonstrated differences in variability between organs and experiments, which was as indicated above accentuated when rates of change were calculated. Furthermore all the rates of change found are not independent values. The final sample of intact plants for any period is also the initial sample for the following period. This means that the estimates of rates of change in successive periods are interdependent, and the significance of their difference must be calculated in a

way different from that of the difference between any two changes more widely separated in time or between changes of different organs. For the difference between two independent estimates of changes, the variance is

$$S_1^2 + S_2^2,$$

where S_1 and S_2 are the standard errors of the two changes in question. For the difference between two *successive* changes in the same organ, however, the variance is:

$$S_1^2 + S_2^2 + S_1 S_2.$$

This expression holds rigidly when the numbers of plants in the three samples are equal, but is a good approximation in other cases.

These various sources of non-orthogonality rendered it well-nigh hopeless to attempt to apply the analysis of variance to these figures.

ACKNOWLEDGEMENTS

I am greatly indebted to Dr. B. D. Bolas, who was largely responsible for the design and construction of the apparatus used and collaborated throughout the work. The arduous labour involved in collecting the data was shared by Dr. Bolas, and occasionally by Dr. I. W. Selman and Mr. D. E. Davies, to whom my thanks are due. In relation to the statistical methods used, consultations with Mr. S. C. Pearce were of the greatest value; I also wish to thank Dr. O. V. S. Heath, Dr. W. L. Stevens, and Dr. F. Yates for advice on particular points. Finally I wish to express my indebtedness to Professor V. H. Blackman and Professor F. G. Gregory for their interest and advice, and to Dr. W. F. Bewley for his encouragement and the ready provision of facilities at Cheshunt for carrying out the work.

The experiments described in this paper formed the basis of a thesis submitted for the degree of Doctor of Philosophy of the University of London in June 1941. Much of the work was carried out during the writer's tenure of a Beit Scientific Research Fellowship.

LITERATURE CITED

- ALEXANDROV, V. G., 1924: La Photosynthèse de différentes feuilles sur une tige de la même plante. *Sci. Pap. appl. Sect. Tiflis bot. Gdn.*, iii. 45.
- ALEXANDROV, W. G., 1928: Über die Assimilations- und Transpirationsarbeit der Blätter der wichtigsten Kachetinschen Weinsorten. *Ber. dtsh. bot. Ges.*, xlv. 126.
- ARTHUR, J. M., GUTHRIE, J. D., and NEWELL, J. M., 1930: Some Effects of Artificial Climates on the Growth and Chemical Composition of Plants. *Amer. Journ. Bot.*, xvii. 416.
- ASHBY, E., and OXLEY, T. A., 1935: The Interaction of Factors in the Growth of *Lemna*. VI. An Analysis of the Influence of Light Intensity and Temperature on the Assimilation Rate and the Rate of Frond Multiplication. *Ann. Bot.*, xlix. 309.
- AUDUS, J. J., 1939: Mechanical Stimulation and Respiration in the Green Leaf. II. Investigations on a Number of Angiospermic Species. *New Phytol.*, xxxviii. 284.
- BALLARD, L. A. T., and PETRIE, A. H. K., 1936: Physiological Ontogeny in Plants and its Relation to Nutrition. I. The Effect of Nitrogen Supply on the Growth of the Plant and its Parts. *Aust. Journ. exp. Biol. med. Sci.*, xiv. 135.

- BLACKMAN, V. H., 1919: The Compound Interest Law and Plant Growth. *Ann. Bot.*, xxxiii. 353.
- BLISS, C. I., 1935: The Calculation of the Dosage-mortality Curve. *Ann. appl. Biol.*, xxii. 134.
- BOLAS, B. D., 1934: The Influence of Light and Temperature on the Assimilation Rate of Seedling Tomato Plants, variety E. S. 1. *Rep. exp. Res. Sta., Cheshunt, 1933*, 84.
- and MELVILLE, R., 1933: The Influence of Environment on the Growth and Metabolism of the Tomato Plant. I. Methods, Technique, and Preliminary Results. *Ann. Bot.*, xlvii. 673.
- and SELMAN, I. W., 1938: The Measurement of Assimilation and Translocation in Tomato Seedlings under the Conditions of Glasshouse Culture. *Ann. Bot., N.S.*, ii. 717.
- BRENCHLEY, W. E., 1920: On the Relations between Growth and the Environmental Conditions of Temperature and Bright Sunshine. *Ann. appl. Biol.*, vi. 211.
- BRIGGS, G. E., KIDD, F., and WEST, C., 1920: A Quantitative Analysis of Plant Growth. Part I. *Ann. appl. Biol.*, vii. 103.
- COMBES, R., 1928: Étude critique de la méthode de Sachs appliquée à la mesure des migrations de substances. *C. R. Acad. Sci., Paris*, clxxxvii. 666.
- 1928a: Influence des traumatismes sur les migrations de substances chez les végétaux. *C. R. Acad. Sci., Paris*, clxxxvii. 993.
- DARROW, G. M., 1932: Methods of measuring Strawberry Leaf Areas. *Plant Physiol.*, vii. 745.
- DAVIS, A. R., and HOAGLAND, D. R., 1928: Further Experiments on the Growth of Plants in a Controlled Environment—I. The Relation of Light Intensity and Exposure Time to Yield. II. The Interrelationship of Temperature and Light. *Amer. Journ. Bot.*, xv. 624.
- DENNY, F. E., 1930: The Twin-leaf Method of Studying Changes in Leaves. *Amer. Journ. Bot.*, xvii. 818.
- 1933: Bases for Calculation in measuring Changes in Leaves during the Night. *Contr. Boyce Thompson Inst.*, v. 181.
- DIJKSTRA, S. J., 1937: Stoffauswanderung und Atmung in Blättern von *Nicotiana glauca* R. Grah. und *Phaseolus multiflorus* Lam. *Rec. Trav. bot. Néerland.*, xxxiv. 333.
- EIDELMAN, Z. M., and BANKUL, E. A., 1933: Effect of Mechanical Reduction of Leaf Area and of Different Alimenta! Conditions upon the Accumulation of Dry Stuff in Cereals. *Bull. Pl. Prot., Leningr., Series III*, iii. 113. (In Russian, English summary.)
- — 1933a: Physiological Value of the Leaves of Different Ages in the Different Stages of the Development of a Given Plant. *Bull. Pl. Prot., Leningr. Series III*, iii. 131. (In Russian, English summary.)
- GOODALL, D. W., 1938: Further Observations on Factors affecting the Position of the First Inflorescence in the Tomato. *Rep. exp. Res. Sta., Cheshunt, 1937*, 73.
- GREGORY, F. G., 1926: The Effect of Climatic Conditions on the Growth of Barley. *Ann. Bot.*, xl. 1.
- GRESSLER, P., 1907: Ueber die Substanzquotienten von *Helianthus Annuus*. *Inaug. Diss., Bonn*. (Quoted by Blackman, 1919.)
- GUTTENBERG, H. v., 1927: Studien über das Verhalten des immergrünen Laubblattes der *Mediterranflora* zu verschiedenen Jahreszeiten. *Planta*, iv. 726.
- HACKENBERG, H., 1909: Über die Substanzquotienten von *Cannabis sativa* und *Cannabis gigantea*. *Beih. bot. Zbl.*, xxiv. i. 45.
- HARRIS, G. H., 1929: Studies on Tree Root Activities: Part II. Some Factors which influence Tree Root Respiration. *Sci. Agric.*, ix. 564.
- HEATH, O. V. S., 1937: The Growth in Height and Weight of the Cotton Plant under Field Conditions. *Ann. Bot., N.S.*, i. 515.
- and GREGORY, F. G., 1938: The Constancy of the Mean Net Assimilation Rate and its Ecological Importance. *Ann. Bot., N.S.*, ii. 811.
- HEINICKE, A. J., and CHILDERS, N. F., 1937: The Daily Rate of Photosynthesis, during the Growing Season of 1935, of a Young Apple Tree of Bearing Age. *Mem. Cornell agric. Exp. Sta.*, 201.
- HENDRICKS, W. A., 1937: The Use of 'Differential Regression' in Analysis of Variance. *Journ. agric. Sci.*, xxv. 256.
- HORNBERGER, R., 1882: Chemische Untersuchungen über das Wachstum der Maispflanze unter Mitwirkung von Raumer. *Landw. Jb.*, xi. 378. (Quoted by Sande-Bakhuyzen, 1937.)
- 1885: Untersuchungen über Gehalt und Zunahmen von *Sinapis alba* an Trockensubstanz und chemischen Bestandtheilen in 7-tägigen Vegetationsperioden. *Landw. Vers.-Stat.*, xxxi. 415. (Quoted by Kidd et al., 1920.)

- INAMDAR, R. S., SINGH, S. B., and PANDE, T. D., 1925: The Growth of the Cotton Plant in India. I. The Relative Growth-rates during Successive Periods of Growth and the Relation between Growth-rate and Respiratory Index throughout the Life Cycle. *Ann. Bot.*, xxxix. 281.
- JOHNSTONE, G. R., 1925: Effect of Wounding on Respiration and Exchange of Gases. *Bot. Gaz.*, lxxix. 339.
- KIDD, F., WEST, C., and BRIGGS, G. E., 1920: What is the Significance of the Efficiency Index of Plant Growth? *New Phytol.*, xix. 88.
- KILLIAN, C., 1933: Recherches écologiques sur l'assimilation chlorophyllienne chez les monocotylédonées printanières. *Rev. gén. Bot.*, xlv. 93.
- KILTZ, H., 1908: Versuche über den Substanzquotienten beim Tabak und den Einfluss von Lithium auf dessen Wachstum. *Inaug. Diss., Bonn.* (Quoted by Blackman, 1919.)
- KOSTYTSCHEW, S., 1921: Studien über Photosynthese. II. Wirkt Wundreiz stimulierend auf die Kohlensäureassimilation am Lichte? *Ber. dtsh. bot. Ges.*, xxxix. 328.
- 1921a: Studien über Photosynthese. III. Findet eine Kohlensäureassimilation während der Sommernächte in der subarctischen Region statt? *Ber. dtsh. bot. Ges.*, xxxix. 334.
- KUDRIAVZEWA, M., MOISSEJEW, W., and SMIRNOVA, M., 1926: Der tägliche Verlauf der Photosynthese bei Landpflanzen. *Planta*, i. 679.
- KREUSLER, U., PREHN, A., and HORNBERGER, R., 1879: Beobachtungen über das Wachstum der Maispflanze. *Landw. Jb.*, vii. 536. (Quoted by Briggs et al., 1920.)
- LUBIMENKO, V., 1923: Influence des blessures des feuilles sur la production de substance sèche chez les plantes vertes. *C. R. Acad. Sci., Paris*, clxxvii. 708.
- MELVILLE, R., 1934: Studies in Growth Relationships in the Tomato Plant: I. Influence of Water Content on Assimilation. Ph.D. Thesis, University of London.
- 1937: The Influence of Environment on the Growth and Metabolism of the Tomato Plant. II. The Relationship between Water Content and Assimilation. *Ann. Bot., N.S.*, i. 153.
- MILLER, E. C., 1917: Daily Variation of Water and Dry Matter in the Leaves of Corn and the Sorghums. *Journ. agric. Res.*, x. 11.
- MONTEMARTINI, L., 1930: Materiali per uno studio del sistema assimilatore delle piante. *Ann. Bot., Roma*, xviii. 38.
- 1933: Alcune osservazioni sopra la produzione e la circolazione degli assimilati nelle piante. *Lav. Ist. bot. Palermo*, iv. 133.
- MUDRACK, F., 1935: Über die Assimilationstätigkeit und das Wachstum von *Ficaria verna*. *Planta*, xxiii. 71.
- MÜLLER, A., 1904: Die Assimilationsgrösse bei Zucker- und Stärkeblättern. *Jb. wiss. Bot.*, xl. 443.
- NAYER, P. P. N., 1936: An Investigation into the Application of Neyman and Pearson's L_1 Test, with Tables of the Percentage Limits. *Stat. Res. Mem.*, i. 38.
- NOVIKOV, V. A., 1936: The Influence of the Intensity of Illumination on the Development of the Cotton Plant. *C. R. Acad. Sci. U.R.S.S.*, ii (i). 397.
- PALMQUIST, E. M., 1938: The Simultaneous Movement of Carbohydrates and Fluorescein in Opposite Directions in the Phloem. *Amer. Journ. Bot.*, xxv. 97.
- PETRIE, A. H. K., WATSON, R., and WARD, E. D., 1939: Physiological Ontogeny in the Tobacco Plant: I. The Drifts in Dry Weight and Leaf Area in relation to Phosphorus Supply and Topping. *Aust. Journ. exp. Biol. med. Sci.*, xvii. 93.
- PORTER, A. M., 1937: Effect of Light Intensity on the Photosynthetic Efficiency of Tomato Plants. *Plant Physiol.*, xii. 225.
- ROODENBURG, J. W. M., 1937: Der Einfluss der Tageslänge im Zusammenhang mit der künstlichen Pflanzenbeleuchtung im Winter. *Ber. dtsh. bot. Ges.*, lv. 5.
- SACHS, J., 1884: Ein Beitrag zur Kenntnis der Ernährungstätigkeit der Blätter. *Arb. bot. Inst., Würzburg*, iii. 1.
- SANDE-BAKHUYZEN, H. L. v. d., 1937: Studies on Wheat grown under Constant Conditions. A Monograph on Growth. Stanford University, California.
- SHIRLEY, H. L., 1929: The Influence of Light Intensity and Light Quality upon the Growth of Plants. *Amer. Journ. Bot.* xvi. 354.
- SMITH, F., 1933: Researches on the Influence of Natural and Artificial Light on Plants. I. On the Influence of the Length of Day. Preliminary Researches. *Meld. Norg. LandbrHøisk.*, xiii. 1.
- STĂNESCU, P. P., 1927: Les Variations quantitatives des produits de la photosynthèse dans

- les feuilles des plantes vertes pendant un jour (vingt-quatre heures). *Ann. sci. Univ. Jassy*, xiv. 383.
- STICHEGLOVA, O. A., and TCHERNYSHEVA, 1933: Studien über den Einfluss der mechanischen Verminderung der Blattfläche auf die Entwicklung der Pflanze, auf den Zuwachs der trockenen Masse und auf die Kornernte bei Sommerweizen und Gerste. *Bull. Pl. Prot., Leningr. Series III*, iii. 73.
- THODAY, D., 1909: Experimental Researches on Vegetable Assimilation and Respiration. V. A Critical Examination of Sachs' Method for using Increase of Dry Weight as a Measure of Carbon Dioxide Assimilation in Leaves. *Proc. roy. Soc. B*, lxxxii. 1.
- TSCHESNOKOV, V., and BAZYRINA, K., 1930: Die Ableitung der Assimilate aus dem Blatt. *Planta*, xi. 473.
- WATSON, D. J., 1936: The Effect of Potassium Chloride on the Diurnal Changes of the Carbohydrate of the Potato Leaf. *Ann. Bot.*, l. 59.
- and BAPTISTE, E. C. D., 1938: A Comparative Physiological Study of Sugar-beet and Mangold with Respect to Growth and Sugar Accumulation: I. Growth Analysis of the Crop in the Field. *Ann. Bot., N.S.*, ii. 437.
- WEST, C., BRIGGS, G. E., and KIDD, F., 1920: Methods and Significant Relations in the Quantitative Analysis of Plant Growth. *New Phytol.*, xix. 200.
- WILLIAMS, R. F., 1936: Physiological Ontogeny in Plants and its relation to Nutrition: 2. The Effect of Phosphorus Supply on the Growth of the Plant and its Parts. *Aust. Journ. exp. Biol. med. Sci.*, xiv. 165.
- 1939: Physiological Ontogeny in Plants and its relation to Nutrition: 6. Analysis of the Unit Leaf Rate. *Aust. Journ. exp. Biol. med. Sci.*, xvii. 123.
- YATES, F. and COCHRAN, W. G., 1938: The Analysis of Groups of Experiments. *Journ. agric. Sci.* xxviii. 556.



An Investigation of the Taxonomic Value of Shoot Structure in Angiosperms with Especial Reference to Leguminosae

BY

K. J. DORMER

(Department of Botany, Queen Mary College, London)

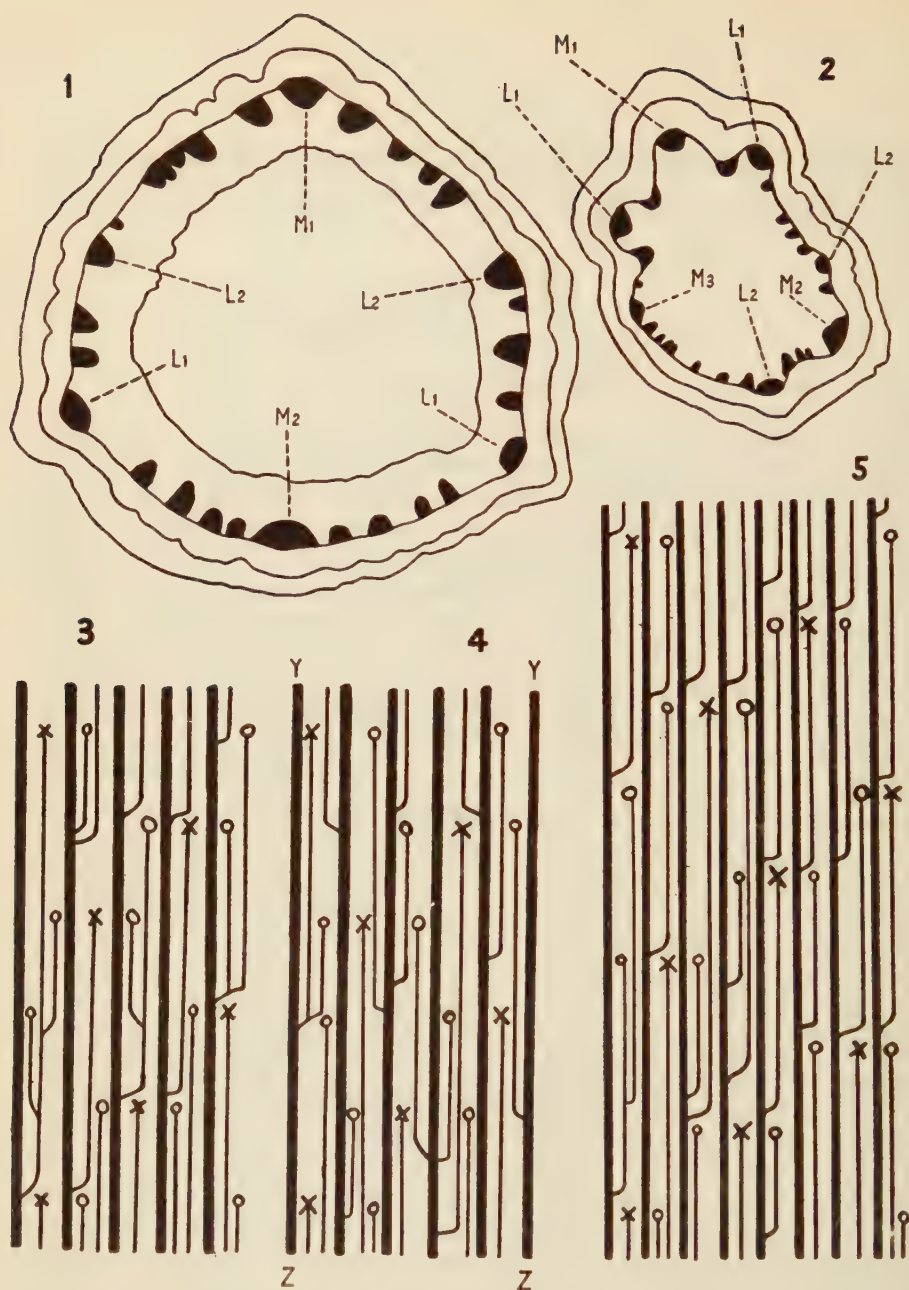
With nine Figures in the Text

THE comparative examination of the vegetative shoots of the Leguminosae was undertaken in an attempt to supply some of the new criteria which Bower (1917) declared to be essential for further progress in the study of angiosperm phylogeny. The purpose of this preliminary paper is to give an outline of those parts of the work which seem likely to have some bearing on the classification of the angiosperms in general, leaving for a future publication such matters as apply more especially to the Leguminosae.

Where tribal groupings are mentioned in the following discussion they are those adopted by Taubert (1891), in the first edition of 'Pflanzenfamilien'.

In view of the prevailing opinion that the vegetative system is too immediately subject to the pressure of physiological necessity to afford characters of more than secondary systematic importance, it may be advisable to begin with some examples in which the internal organization of the shoot displays a good deal of rigidity in spite of conspicuous modifications in external appearance. The erect aerial shoots of *Thermopsis montana*, a perennial herb, bear distichously placed leaves, each of which is supplied by three trace bundles. The disposition of the traces as seen in a transverse section of the stem is shown in Fig. 1. This arrangement is preserved in the subterranean rhizome, although the histological differences between the two organs are considerable. *Gleditschia sinensis* is a large tree, the twigs of which bear large bipinnate leaves placed spirally and each supplied by three traces. The bundles supplying the axillary branch arise, as in all Leguminosae, from the margins of the gap associated with the median leaf-trace. The arrangement of the bundles as seen in a transverse section of the stem is shown in Fig. 2. Besides the normal twigs there are reduced branch-systems in which each axis ends in a spine, the leaves being represented by mere scales. In this case as in *Thermopsis* the general layout is the same in the modified shoots as in the normal ones, though here also there are conspicuous histological differences.

Apart from such instances as these, however, the taxonomic value of the characters here considered is amply demonstrated by the tendency for the same type of structure to run through a whole genus or series of genera, and there can be no doubt that phyllotaxy and the arrangement of the primary



FIGS. 1-5. 1. Transverse section of aerial shoot of *Thermopsis montana*. M₁ and L₁, the median and lateral traces of the first leaf above the plane of section, M₂ and L₂, those of the second leaf above. 2. Transverse section of a young internode of *Gleditsia sinensis*. M₃, median of third leaf above. 3. Reconstructed vascular system of *Acacia Baileyana*. Stele supposed to be opened down one side and then laid out flat. Crosses represent median, and circles lateral traces. 4. Vascular system of *Sophora tetraptera*. Bundle YZ is represented twice over. 5. Vascular system of *Sutherlandia frutescens*.

vascular system are capable of playing an important part in any phylogenetic treatment of the family.

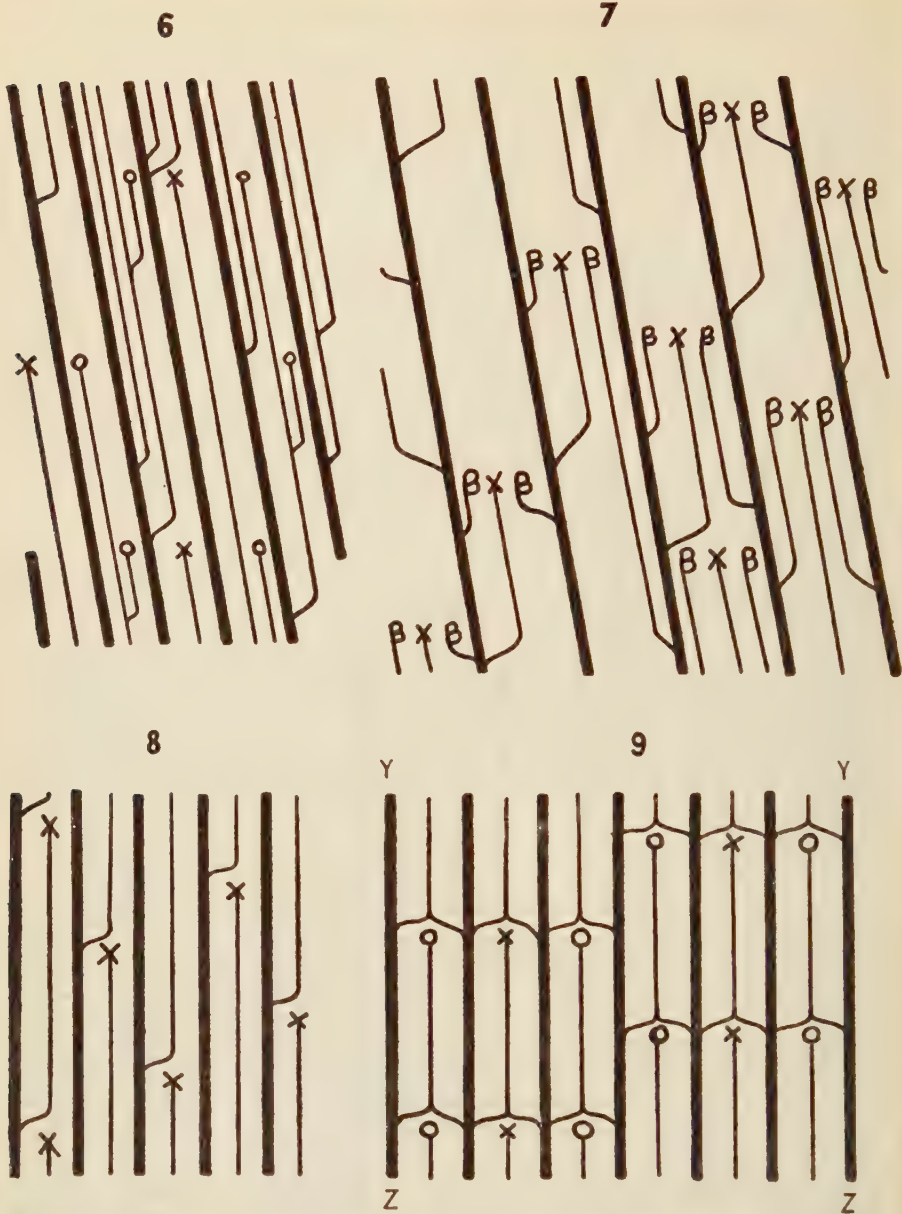
The form of shoot found in *Acacia Baileyana* appears to be the basic type for the Leguminosae. The stem is woody, and bears bipinnate leaves in a regular $2/5$ spiral. There are no obvious stipules, though some closely allied species possess well-developed stipular spines, but at the base of each petiole there is a distinct pulvinus. If the primary xylem system of the stem is reconstructed from serial sections, the result is as shown in Fig. 3, in which the stele is represented as being opened down one side and then laid out flat. It is clear that the stem contains five bundles which are indefinitely prolonged upwards, and that these stem bundles give off as branches the traces to the leaves. Each leaf receives three traces, the median being denoted by a cross, the laterals by small circles. Between each lateral trace and the corresponding median one a single stem bundle is included. In consequence of this arrangement, the katadromic lateral of each leaf is superposed to the anadromic one of the leaf below. If the shoot were seen in plan view, the insertions of successive leaves would appear just to touch.

In this particular example all the traces depart from the stem bundles in the same direction, and they are often fused with one another in the lower parts of their courses. These, however, are points of minor importance. The significant features are the spiral phyllotaxy, the absence of any anastomoses in the vascular system of the stem, the inclusion of a single stem bundle only between the median trace and each lateral, and the way in which the insertions of successive leaves just meet as seen in plan view. Any shoot which exhibits these characteristics may conveniently be described as acacian. Thus *Sophora tetraptera*, the vascular system of which is shown in Fig. 4, is a quite typical acacian form.

The simple acacian plan is subject to a variety of modifications. Fig. 5, which was drawn from a shoot of *Sutherlandia frutescens*, shows a case in which there are eight stem bundles, the leaves being placed in a spiral with a divergence of $3/8$. Sometimes the number of orthostichies does not equal the number of stem bundles, and then the bundles necessarily pursue a helical course. This is well seen in the shoot of *Mimosa Spegazzinii* (Fig. 6) and in the lateral spinescent shoot of *Ulex europaeus* with irregular phyllotaxy (Fig. 7). In this last example, only the median trace of each leaf is developed, as is also the case in *Cytisus scoparius*, Fig. 8.

The acacian shoot, either in its typical form or with such obvious modifications as those just described, is found throughout the Mimosoideae, in most Sophoreae and Genisteae, and in the Australian and South African genera of Podalyriaceae. It occurs also in a number of other Papilionatae, but is not yet known from the Caesalpinioideae, nor has it been found in any herbaceous species. The restriction of the acacian type of shoot to woody forms is of great interest, but cannot profitably be discussed until the structure of some typical herbs has been described.

Fig. 9 was drawn from a species of Ornithopus, but will serve, with slight



FIGS. 6-9. 6. Vascular system of *Mimosa Spegazzinii*. 7. Ditto, *Ulex europaeus*. B, branch trace. This is one of the short lateral spinescent shoots in which the phyllotaxy is often very irregular. 8. Vascular system of *Cytisus scoparius*. 9. Ditto, *Ornithopus sativus*, the bundle YZ represented twice.

alterations, for a considerable range of mainly or entirely herbaceous genera. It is clear that this vascular system differs from all those previously considered in the presence of regular anastomoses which unite the whole of the primary xylem of the stem into a continuous network, in place of the separate branching bundle-systems of the acacian shoot. Any vascular system in which such anastomoses occur will here be described as a closed system, while those from which they are absent may be distinguished as open systems.

Closed systems of one kind or another are characteristic of Loteae, Coronilinae, Viciae (except Abrus), the northern genera of Podalyrieae, and most Trifolieae, besides occurring in a number of other Papilionatae and a fair proportion of the Caesalpinoideae. No closed system is known from the Mimosoideae. Closed systems are most usually associated with distichous phyllotaxy, though a number of spiral closed types are known, but they are not by any means confined to herbs. In particular there are several tribes of Caesalpinoideae which are almost entirely woody with closed systems.

It is clear that in a shoot with an open vascular system the primary vascular tissues are broken up into discrete units, which communicate with each other only indirectly through the leaves. In a woody plant any physiological effect which this separation might have is presumably nullified at an early stage by the formation of a continuous cylinder of secondary tissues. In a typical herb, on the other hand, it seems, in view of the known physiological importance of the tissues involved, not unreasonable to suppose that the lack of tangential continuity in the primary xylem and phloem would result in a certain amount of functional inefficiency. When the matter is seen from this point of view, the prevalence of closed systems among the main herbaceous groups becomes intelligible.

The simplicity of the situation is somewhat marred by the existence of a great number of herbs with open systems. Open systems are the rule, for example, among the Phaseoleae. These are specialized open types, far removed from the simple acacian plan, but the same considerations as to tangential discontinuity must be supposed to apply. The apparent discrepancy is resolved by the fact that in all these herbaceous open types a continuous vascular cylinder is developed at an early stage of growth. Such a herb is to all intents and purposes a woody plant which only lives to produce a single annual ring, and is not to be confused with the extreme herbaceous type in which interfascicular xylem and phloem are laid down late or not at all. No such typical herb has been found with an open system.

Owing to their greater complexity, the specialized open types mentioned in the preceding paragraph have not been analysed in detail, and it will be sufficient for the present purpose to give a few examples to show how they depart from the acacian scheme. It is clear that in the shoot of *Gleditschia* shown in Fig. 2 the insertions of successive leaves are widely separated as seen in plan view, instead of being in contact as in an acacian shoot. Such a separation occurs constantly in the Dalbergieae and Robiniinae, besides appearing sporadically elsewhere, and may be much more pronounced than

in the example illustrated here. Where the vascular system is of this type, the phyllotaxy is often subject to strange disturbances. This is most clearly seen in *Lespedeza Sieboldi*, in which the arrangement of the leaves appears to be governed solely by the consideration that two cannot arise at the same place. Both the angle of divergence and the length of the internode vary widely and at random. A limiting condition seems to be reached when two leaves stand at the same level, and so close together that their adjacent lateral traces are completely fused for the greater part of their length, only separating as they run out through the cortex. A similar state of affairs has been observed in *Erythrina Crista-galli*.

When the leaf-base is very wide, as in the *Thermopsis* shown in Fig. 1, the insertions may be said to be interlocked. This particular example happens to be a closed system, but the same kind of layout is often found in open systems among the Papilionatae, and rather less frequently among the Caesalpinioideae. Viewing the family as a whole, it appears that the types of insertions are distributed without reference to phyllotaxy, and regardless of the distinction between open and closed systems.

Lastly, it is necessary to devote some attention to the multilacunar forms. The work of Naegeli (1858), Sinnott (1914), and Watari (1934) leaves no doubt that the number of traces to each leaf in this family is prevailingly three, though both larger and smaller numbers occur in many species. It is striking that such departures from the trilacunar state are only known in open systems. This can hardly be accidental, and is perhaps a consequence of the greater standardization of the closed system. Reference to the diagrams will show that in an open system the addition or omission of traces need not involve any other alteration, whereas in a closed system no change in the number of traces can be effected without profoundly modifying the original symmetry. Multilacunar nodes are particularly frequent among the Eucaesalpinieae, Phaseoleae, and Desmodiinae, besides occurring sporadically elsewhere in the Papilionatae. It is probable that several distinct types exist, the multilacunar state having been attained by different methods. In some instances it seems almost certain that supernumerary traces have been added on the flanks of the original trilacunar insertion, while in others there appears to have been a diversion into the leaf of some of the traces normally destined to serve the axillary branch.

The nature of the available data having been explained, it remains to discuss their application to problems of phylogeny.

Set out in Table I are a number of pairs of contrasting characters, one member of each pair being regarded as primitive, the other as relatively advanced. It is impossible to give in full the evidence on which this table is based, but some examples will show the kind of reasoning employed.

There are in the Galegeae two closely allied sub-tribes, the Coluteinae and the Astragalinae, which have in common not only many floral characters but also a type of stipule not known anywhere else in the family. Beginning with the Coluteinae, we find the acacian shoot in its typical form in Colutea,

a small genus of Mediterranean shrubs. At the Cape, a modified acacian type appears in *Sutherlandia*, a monotypic shrub. Besides the spiral shoots, one of which is shown in Fig. 5, this species also produces a few twigs with distichous phyllotaxy. Also at the Cape is the large genus *Lessertia*, which is

TABLE I

Reference number.	Primitive characters.	Advanced characters.
1	Phyllotaxy spiral	Phyllotaxy verticillate or distichous
2	Woody plant	Herb
3	Open system	Closed system
4	Insertions in contact	Insertions separated or interlocked
5	Leaf highly compound	Leaf less compound
6	Stipules present, free	Stipules absent or adnate to petiole
7	Leaf with three traces	Leaf with more or less than three traces
8	Pulvinus present at base of petiole	No general foliar pulvinus.

predominantly distichous, though a small proportion of the species regularly produce spiral shoots. Whereas the preceding genera are entirely woody, many species of *Lessertia* are herbaceous. Lastly, in Australia and New Zealand, the group is represented by *Clianthus* and *Swainsona*, which appear to be entirely distichous, with a high proportion of herbaceous species, and with closed systems not essentially different from that shown in Fig. 9. Clearly in this group there is a regular gradation in structure, though there is nothing to show which way the series is to be read. All doubt on this point seems to be removed by a consideration of the nearly related *Astragalinae*. Here the acacian type is represented by two Mediterranean genera, *Caragana* and *Halimodendron*. The remainder of the group is composed of partly or entirely herbaceous genera, with closed systems, and displaying much greater diversity of structure than the *Coluteinae*. These closed types are scattered almost all over the world. The simplest interpretation is that both sub-tribes sprang from an acacian stock centred in the Mediterranean area, the more specialized derivative herbaceous forms having spread widely over the rest of the world. This view derives further support from the existence of very similar relationships among the *Trifolieae*, where the acacian type is represented by the Mediterranean *Ononis fruticosa*, while the herbaceous genera with closed systems are generally distributed. It will be seen that this interpretation accords with the views expressed in Table I.

Another line of argument is that based on the distribution of characters among the sub-families. Thus closed systems only occur in the *Papilionatae* and *Caesalpinioideae*, which suggests that they are more specialized than the open type. Similarly, bipinnate leaves are almost universal among the *Mimosoideae*, occur in perhaps about half the *Caesalpinioideae*, but are unknown in *Papilionatae*, which is in itself a reason for supposing the more

highly compound type of leaf to be primitive. Again, although exact figures are not available, it is certain that the proportion of species with distichous phyllotaxy to those with spiral is lowest in the Mimosoideae, which is in agreement with the view that distichous phyllotaxy is derived from spiral rather than the converse.

Now if the conclusions stated in Table I are correct, it is to be expected that when allied forms differ in more than one of the characters considered, the differences will, on the whole, tend to be correlated. That is to say, forms which are specialized in one respect should also be specialized in others. Numerous examples of such correlation have come to light, only a few of which will be mentioned here.

Of the groups which have already been considered in some detail, it is clear that the Coluteinae show correlation between the characters numbered 1, 2, and 3. In the Astragalinae and Trifolieae, where the genera with closed systems show interlocking of the insertions, there is a correlation also with character 4.

Correlation on a more extensive scale is to be observed in the Caesalpinioideae, where the tribes with bipinnate leaves are entirely spiral, while those with simply pinnate leaves are largely distichous. The Bauhinieae, in which the reduction of the leaf has proceeded farthest, are entirely distichous. The correlation in this case is between characters 1 and 5.

As an example of correlation at the level of species, the genus *Desmodium* is particularly instructive. The genus is a large one and is present in all tropical countries and many extra-tropical ones. The vascular system is uniformly open, but is otherwise very variable. Each leaf may have three leaflets or only one. In order to express the variation in the proportion of unifoliate species in different countries, the procedure has been adopted of calculating, from various published floras, the average number of leaflets possessed by the species found in the area covered. A few species in which the leaves are variable were treated as unifoliate. Such a treatment is perhaps arbitrary, but in any case there were not enough ambiguous cases to make any important difference to the results. In Table II the countries covered by the various

TABLE II

Leaflet averages.	Countries covered by floras used.
3.00	Canada and Northern U.S.A.
2.86	South-eastern U.S.A.
2.86	Panama Canal Zone
2.80	Jamaica
2.54	West Tropical Africa
2.50	Brazil
2.47	Malay Peninsula
2.43	British India
2.41	Tropical Africa
2.33	City of Madras
2.30	Ceylon
2.28	Australia
2.20	Sudan
2.20	Transvaal, with Swaziland.

floras used are placed in numerical order of averages, and it is clear that the values for the New World are significantly higher than those for the Old World, indicating that the American forms are relatively primitive. Regarding phyllotaxy, such precise data are unfortunately not available, owing to the neglect of this character in floristic works. An estimate based on the Indian species shows that about 70 per cent. are distichous, the remainder being spiral. A survey of the available American material makes it highly improbable that the proportion of distichous species for the New World as a whole will exceed 10 per cent., and it may well be less than 5 per cent. Many, though by no means all, the Asiatic species are multilacunar, with insertions that occupy more than half the circumference of the stem, while the American species, so far as present knowledge goes, are all trilacunar, mostly with relatively narrow insertions. In this genus, therefore, there is clear evidence of correlation between characters 1, 5, and 7. When the bearing of this work on the classification of the Leguminosae comes to be considered in a later paper, it will be shown that there is some external evidence for the view that the genus *Desmodium* originated in the American continent, and this obviously agrees with the indications within the genus itself.

The question now arises how far the criteria which we have been considering, and which are undoubtedly of the greatest importance within the Leguminosae, are likely to prove applicable also to other groups. The indications on this head are encouraging so far as they go. The fullest information available is that concerning phyllotaxy. In considering the significance of the distichous or dimerous type of shoot, it will not be necessary to distinguish between the spiral forms and the verticillate and decussate ones, all of which may conveniently be bracketed together as polymerous. Some families are entirely dimerous, as for example Anonaceae, Betulaceae, Myristicaceae, Orchidaceae, and Ulmaceae, while others, such as Anacardiaceae, Araceae, Bromeliaceae, Cruciferae, and Thymelaeaceae appear to be exclusively polymerous. In the present connexion, however, the greatest interest attaches to the mixed families.

In Table III are set out some of the more important floral characters of the

TABLE III

Floral features.	Phyllotaxy.	Genera.
Flowers hermaphrodite	Mainly polymerous	Royena
Dioecious		
All stamens fertile	Polymerous	Euclea
Staminodes present	Mostly dimerous	Maba and Diospyros

principal genera of Ebenaceae. It should be clear that the genera which display the greatest floral specialization are also those which possess what we are supposing to be an advanced type of vegetative shoot.

Table IV summarizes the data available for the Hamamelidaceae, the main divisions adopted being those of the 'Genera Plantarum' of Bentham and Hooker, though they have been rearranged so as to bring the more primitive forms to the beginning of the table.

Table V deals with the Bixaceae of Bentham and Hooker, which includes the Flacourtiaceae of modern authors.

Table VI covers the Tiliaceae of Bentham and Hooker, with the exception of the genera now separated as the Elaeocarpaceae. In this instance the

TABLE IV

Floral characters.	Phyllotaxy.			
	No. of genera.	No. seen.	Poly.	Di.
Loculi with numerous ovules	4	3	3	—
Loculi with a single ovule				
Petals present	7	5	1	4
Petals absent	4	4	—	4

TABLE V

Floral characters.				No. of genera.	No. seen.	Poly.	Mixed.	Di.
Anthers linear	Hermaphrodite	Fruit capsular	Corolla present, without scales	3	2	2	—	—
Anthers short, globular	Polygamous	Fruit mostly baccate		4	3	3	—	—
Ditto	Largely dioecious	Ditto.	Often apetalous	15	13	1	6	6
Ditto	Dioecious	Ditto	Corolla with scales	7	6	3	—	3

TABLE VI

Fruit characters.	No. of genera.	No. seen.	Poly.	Mixed.	Di.
Completely dehiscent	32	26	6	2	2
Indehiscent or sometimes opening slightly at the tip			2	2	12

tribal divisions are not convenient for the present purpose, as they are not of a nature which facilitates the discrimination of primitive and advanced types. Accordingly, the genera have been grouped on a basis of fruit structure.

It is clear that in these examples there is a marked correlation between advance in floral structure and advance in vegetative structure. The tables may profitably be read in conjunction with the list of characters given by Hutchinson (1926).

The view that verticillate phyllotaxy is derived from spiral is now almost a commonplace. (See Hutchinson, 1926.) Nevertheless, it may be advisable to give one or two examples of this kind of progression, as the characters of opposite or alternate leaves have only been extensively used by systematists in those instances where they happen to be constant through large taxonomic units such as families. We shall find that these features are not to be neglected even where they are quite inconstant.

Table VII was drawn up from the account of the Loasaceae given in the first edition of 'Pflanzenfamilien'. In this, as in the following instance, no attempt has been made to discover whether any distichous types occur, but it may be taken for granted that the genera with alternate leaves are for the most part spiral.

TABLE VII

Floral characters.				Phyllotaxy.		
				Alternate.	Mixed.	Opposite.
Stamens few	.	.	.	2	—	—
Stamens numerous, in bunches like those of <i>Hypericum</i>	.	.	.			
Stamens all fertile	.	.	.	1	2	—
Staminodes present	.	.	.	1	1	5

TABLE VIII

Floral characters.			Phyllotaxy.		
			Alternate.	Mixed.	Opposite.
Stamens 4-theous	Several ovules	Ovules erect	5	—	—
Ditto	Single ovule	Ditto	11	—	1
Ditto	Ditto	Ovules pendulous	5	6	22
Stamens 2-theous	Ditto	Ditto	1	—	12

Table VIII is based on the arrangement of Amarantaceae in the second edition of 'Pflanzenfamilien', and is not quite complete, the phyllotaxy of one genus in the last group not being stated.

The Hydrocharitaceae are of interest in that the marine genera show specialized phyllotaxy, *Enhalus* and *Thalassia* being distichous, and *Halophila* exhibiting a peculiar arrangement with leaves in opposite pairs, successive pairs being almost superposed. Apart from the verticillate arrangement seen in the submerged aquatics *Hydrilla* and *Elodea*, the rest of the family are spiral.

Regarding the other features of shoot morphology which have been considered, such as the distinction between open and closed systems and between the various relations existing among the traces of different leaves, very little can be said. Almost the only available information is that contained in the classical paper by Naegeli (1858), which is quite insufficient to form the basis of any general treatment. It may, however, be worth while to point out that some of Naegeli's findings are not inconsistent with the view that closed systems have been derived from open ones. Thus it appears that the Abietaceae are open, while the Cupressaceae are closed. It will be noted that this difference is correlated with a difference in phyllotaxy. The diagrams given at least serve to show that the distinction between open and closed systems is

likely to be applicable to a considerable range of angiosperms and gymnosperms.

The number of traces serving each leaf has already been dealt with by Sinnott (1914), whose conclusions as to the relatively primitive nature of the trilacunar node agree with the view expressed in Table I of the present paper. The view that the woody shoot is more ancient than the herbaceous type has already been reached by Bailey and Sinnott (1914) and by Hutchinson (1926), who makes the point a principal feature of his classification of the Dicotyledons. It seems therefore to be unnecessary to bring further evidence for these interpretations.

In conclusion, it may be pointed out that there is, in the present state of taxonomic botany, a great wastage of opportunity due to the neglect of some of the features mentioned here. Phyllotaxy, which is determined almost at a glance, offers perfectly good sectional characters in a number of difficult genera like *Desmodium* and *Cassia*. It would probably be useful in *Ononis*, but this is one of the many instances where phyllotaxy, though obvious in fresh material, is indeterminable from herbarium sheets. The same may be said of the *Commelinaceae*. It is clearly desirable that anyone who describes a plant from fresh material should state the phyllotaxy, particularly in the case of herbs or of any plant with abnormally long internodes. This is all the more important as the arrangement of leaves is often misrepresented even in the best of published plates. Similarly, a section through a node, prepared and examined in precisely the way which is now traditional for ovaries, will give information quite as important, and quite as difficult to get once the specimen has been dried.

SUMMARY

It is found that phyllotaxy and the arrangement of the primary vascular system are of value in the taxonomic and phylogenetic treatment of the Leguminosae. Contrasting pairs of primitive and advanced features are set out in Table I, and examples are given to show how the evolution of these characters has been correlated, so that forms which are specialized in one respect are specialized also in others. The taxonomic value of phyllotaxy is shown to extend to many other angiosperm families.

It is suggested that the evolution of a typical herbaceous habit becomes possible only when the tangential continuity of the vascular system is maintained by nodal anastomoses between the bundles, so that there is no necessity for the formation of a continuous cylinder of secondary tissues.

The greater part of this work was carried out during tenure of Postgraduate Studentships of Queen Mary College and the University of London. I wish to record my gratitude to Professor F. E. Fritsch for advice and assistance, and to the Director of the Cambridge Botanic Garden who supplied the material for investigation.

LITERATURE CITED

- BAILEY, I. W., and SINNOTT, E. W., 1914: The Origin and Dispersal of Herbaceous Angiosperms. *Ann. Bot.*, xxviii. 547.
- BOWER, F. O., 1917: The Natural Classification of Plants. *Journ. Linn. Soc.*, xlv. 107.
- HUTCHINSON, J., 1926: Families of Flowering Plants, vol. I. Dicotyledons. London.
- NAEGELI, C., 1858: Ueber das Wachstum des Stammes und der Wurzel bei den Gefäßpflanzen. *Beit. zur Wiss. Bot.*, i.
- SINNOTT, E. W., 1914: The Anatomy of the Node as an Aid in the Classification of Angiosperms. *Amer. Journ. Bot.*, i. 303.
- TAUBERT, P., 1891: Leguminosae in Engler and Prantl's *Pflanzenfamilien*. Edit. I Berlin.
- WATARI, S., 1934: Anatomical Studies on some Leguminous Leaves with Special Reference to the Vascular System in Petioles and Rachises. *Journ. Fac. Sci. Tokyo*, iv. 225.

Chromosome Length at the Early Meiotic Prophases in *Osmunda*

BY

IRENE MANTON

(*Botany Department, University of Manchester*)

With Plates I and II and forty-nine Figures in the Text

	PAGE
INTRODUCTION	155
MATERIAL AND METHODS	156
MORPHOLOGY OF THE EARLY PROPHASE STAGES	157
ZYGO-PACHYTENE MEASUREMENT IN ACETOCARMINE	159
MEASUREMENT AT LEPTOTENE IN A SECTION	160
OBSERVATIONS ON 'EARLY' PACHYTENE	161
COMPARISON OF PACHYTENE PAIRING IN THE POLYPLOID SERIES	167
SUPPLEMENTARY OBSERVATIONS ON SHRINKAGE AT 'LATE' PACHYTENE	170
SUPPLEMENTARY OBSERVATIONS ON THE SUPERCONTRACTED LENGTH	172
CONCLUSIONS	174
SUMMARY	175
LITERATURE CITED	176

INTRODUCTION

IN a previous paper on spiral structure and chromosome pairing in *Osmunda regalis* L. (Manton, 1939) two critical observations were shown to be in need of further evidence. By the elaboration of special methods one of these observations (concerning the number of coils in a somatic chromosome) has since been fully confirmed (Manton and Smiles, 1943). It is the aim of the present paper to fulfil a like service for the other.

The previous evidence on the length of a chromosome at its fullest extension had consisted, essentially, in one direct measurement of a complete chromosome at leptotene, obtained from an acetocarmine squash made permanent by McClintock's method. Apart from the obvious dangers of using a unique specimen as principal evidence, the observation was uncertain, not from any defect in the specimen itself, which was an unusually perfect one, but from a difficulty inherent in the technique used. Acetocarmine, though of inestimable value for flattening and spreading a nucleus thereby making possible an observation that could not otherwise have been made, was known to have the very serious disadvantage (in this particular context) of materially altering chromosome size. At all stages at which comparable measurements could be made by other methods the apparent dimensions in acetocarmine prepara-

tions were found to be almost double those obtained in sections or smears. This enlarging effect is well known (cf. for example Manton, 1937; Sparrow, Huskins, and Wilson, 1941) and no obvious other distortion appears to accompany it. In its mechanism it is perhaps to be compared to the reversible enlargement produced by hot acetic acid on the proteinaceous textile fibres (cf. Speakman, 1931). For many cytological purposes it is of great use in making visible details which would otherwise be too small to be seen, but for measurements of length the difficulties introduced are obvious. Not only does collation of results with those from other techniques involve much awkward cross-reasoning, but the risk cannot be wholly excluded that the artifact might differ, selectively, at different stages of the chromosome cycle and in that case the observations might be seriously misleading.

It may be said at once that this has been found not to be the case. By altering the mode of approach to the problem the technical methods previously used have been made to yield confirmatory evidence, both direct and indirect, on a scale sufficient to authenticate fully the validity of the original observation.

The plan of inquiry has been to study intensively the early meiotic prophase in diploid, triploid, and tetraploid plants with the object of obtaining at least ten whole chromosomes at the earliest workable stage in each. In the light of previous experience of the extreme difficulty of working leptotene directly, attention has been primarily centred on early pachytene; nevertheless one fortunate example of a measurable chromosome at leptotene in a section has been obtained. At pachytene itself some fifty whole chromosomes have been detected and figured, all of which are at stages considerably earlier than those previously worked. Most of these figures are reproduced and a proportion of them are authenticated by photographs; the detailed measurements are contained in the tables. The body of data thus supplied is sufficient to settle the main point at issue with some degree of finality and a gratifyingly simple relation is found to exist between the length of a chromosome at its fullest extension and at other significant stages of the nuclear cycle. In addition, the comparison of the details of pachytene pairing in the various polyploid plants is of some interest for its own sake.

MATERIAL AND METHODS

The plants used have been well-matured specimens of diploid, triploid, and tetraploid *O. regalis* long established in Manchester and the source of previous cytological material. The only other point worthy of emphasis is that the series is a strictly autopolyploid one, having arisen (see Manton, 1932; Lang, 1924) as a consequence of induced apospory in a normal plant.

Since it was essential to the problem that the technical methods should be comparable with those previously used, little need be said about them. Sections fixed in 2 BD, cut at 14–16 μ , and stained in haematoxylin have yielded the principal evidence, the only variation on the earlier procedure being the frequent dilution of the fixative with half its volume of water and the intro-

duction of a light counterstain with bismarck brown to show up cytoplasm and cell walls. The details of the acetocarmine squash technique, which has been sparingly employed, are exactly as in 1939.

One technical improvement on previous methods has been made with regard to the measurement of selected chromosomes. A prophase chromosome is an intricately contorted thread of considerable length. The rather cumbersome methods of measurement enumerated in an appendix to the previous paper have now been superseded by the following procedure. The outline of the selected chromosome is projected on paper by photography or other means. In the most favourable cases photographs were actually taken and an enlarged print prepared on matt-surfaced bromide paper; in other cases the preparation was put on the photographic apparatus as if for an exposure and a pencil outline was drawn upon a piece of transparent paper placed over the image on the ground glass, a high magnification (3,000 or 4,000) being employed. The outline, however obtained, is then made the basis of a careful drawing and the drawing when finished is measured with a pair of needle-pointed callipers opened to any selected extent, 2 mm. and 5 mm. being the most usual intervals. The callipers are then moved down the outline, if necessary perforating the paper with the needle-points at every step. The number of steps multiplied by the distance between the points is then the length required. This method of measurement is simple and rapid and appears to be the most accurate of any yet tried.

MORPHOLOGY OF THE EARLY PROPHASE STAGES

Before the collection of data could be begun it was necessary to reconsider to some extent the seriation of stages and to subdivide certain of these, notably pachytene. In compiling the sequence of photographs of the mitotic and meiotic cycles previously published (Manton, 1939) the general text-book definitions of the stages had been utilized with only minor emendations. Thus it had been found convenient to insert a 'preleptotene' as a separate stage from 'leptotene' but pachytene was merely subdivided into 'early' and 'late'. This treatment of pachytene must now be regarded as inadequate and at least three stages can profitably be distinguished, which may provisionally be referred to by the descriptive epithets of 'polarized pachytene', 'spread pachytene', and 'contracting pachytene'. The last of these is the 'late' pachytene of the previous paper. The 'early' pachytene of that work is a slightly imperfect example of spread pachytene, but polarized pachytene was not represented at all. Its characteristics will at once be apparent by a glance at Pl. I, Fig. 22, which contrasts strikingly with Pl. I, Fig. 24, representing spread pachytene on the same preparation. The omission of the former stage from the previous work was no doubt in part the result of a prejudice against the synizetic appearances, since there is a widespread hypothesis that these are artificial. It has, however, become increasingly apparent that to delete all preparations showing such cells will merely result (in this organism at any rate) in the

exclusion of a part of the nuclear cycle from study. This can be all too easily done if detached cells in smear preparations are exclusively used. In sections of pinnae bearing large numbers of sporangia such a procedure, however, is impossible without doing violence to the evidence; and since some of the clearest chromosome measurements have in fact been obtained from strongly synizetic cells in preparations which also showed perfect examples of the later stages, the interpretation of synizesis as 'chromosome shrinkage' may have to be reconsidered.

In defining the stages, attention has been given to a larger number of criteria (involving observations of cell walls, cytoplasm, state of the tapetum, &c.) than were previously used. Even so, it need hardly be pointed out that the significant differences between stages are only very superficially known and rarely, if ever, is it possible to draw sharp lines of demarcation between them. This is particularly true of the transition from zygotene to pachytene and from 'spread' to 'contracting' pachytene. If cognizance could be taken of criteria such as the cessation or onset of chromosome movement or of changes in their minuter structure, it is possible that the beginnings of these stages, as well as their significance, might be more clearly discernible. In the absence of such knowledge one must recognize that the criteria which lend themselves to observation for the diagnosis of stages may in themselves be only incidental accompaniments to more important happenings.

Without prejudging the extent to which the situation in *Osmunda* will be found to apply to other organisms, it may be of convenience to the reader, both in understanding the new data and in collating it with the old, if a brief synopsis of the meiotic prophase stages now recognized in this plant be given. This will summarize in advance some of the more general results of the new observations and may at the same time put the new stages into their proper context. The series of early prophase stages in their present emended form may therefore be listed as follows:

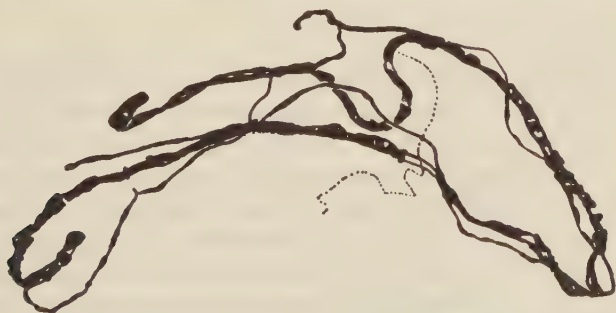
1. *Rest*. 'Solid' nucleus with several nucleoli. Chromosomes not individually distinguishable. Figured Manton, 1939.
2. *Preleptotene*. Nucleus densely filled with coiled chromosome threads, the coils presumably relic coils from the spirals of the previous division; chromosome length unknown. Figured Manton, 1939.
3. *Leptotene*. Nuclear diameter increased by accumulation of nuclear sap. Synizetic appearance beginning. Displacement and coalescence of nucleoli suggests onset of chromosome movement. Chromosomes fully elongated, free from relic coils, unpaired but much entangled. Figured Manton, 1939.
4. *Zygotene*. Lateral pairing of chromosomes in progress. Some degree of synizetic appearance always displayed. Figured Manton, 1939.
5. *Polarized pachytene*. Synizetic appearance at its fullest development. The symmetry of the whole cell is affected; the nucleus is no longer centrally placed and the position of the 'synizetic knot' is always on the side of the nucleus nearest to the cell surface and away from the bulk

of the cytoplasm; the plane of symmetry of adjacent cells is at random relative to each other. Chromosomes are fully elongated and stain intensely; they are as completely paired as they will be. Not figured in 1939, but shown here in Pl. I, Figs. 1, 5, 16, 22.

6. *Spread pachytene*. Synizetic appearances effaced. Paired chromosomes evenly filling the nuclear cavity suggest a passive flotation out from the entanglement of earlier stages consequent on cessation of the pairing movements. Chromosomes still fully elongated. Imperfectly figured in 1939 as 'early pachytene'; figured here in Pl. I, Figs. 6, 7, 8, 11, 24, &c.
7. *Contracting pachytene*. Somewhat resembling the last in general appearance but chromosomes shrinking. Mother cells usually loosening apart from each other and from the sporangium wall. Walls of tapetal cells beginning to break down, and their contents beginning to intrude between the separating mother cells. An advanced state figured as 'late pachytene' in 1939; figured here in Pl. I, Figs. 9, 10, 21.
8. *Strepsitene*. Chromosome shrinkage (= supercontraction) apparently complete. Chiasmata not yet revealed but chromosome separating. Figured 1939.
- 9 and 10. *Diplotene* and *Diakinesis* in the usual sense of the terms. Figured 1939.

ZYGO-PACHYTENE MEASUREMENT IN ACETOCARMINE

The only new measurement that will be quoted from an acetocarmine preparation is that represented by Pl. II, Figs. 27 and 28 and Text-fig. 1.



TEXT-FIG. 1. Trivalent at late zygotene or early pachytene in autotriploid *Osmunda* (plant II), from a permanent acetocarmine preparation. ($\times 3,000$.) Photographs in Pl. II, Figs. 27-8, description in text.

The specimen is from a triploid plant and is a complete trivalent at a very early stage of pairing. It is not easy, in the detached condition of a squash, to determine whether the sporangium is actually in zygotene or in the immediately following stage of polarized pachytene, but the latter is the more probable.

The length of this specimen is approximately 210 μ m. at the magnification

of the text-figure or, reduced to 1,000 diameters, 70 mm.¹ Now it may be recalled that the measurement previously published (Manton, 1939) for a whole chromosome at leptotene in this technique and at a magnification of 1,000 was 73 mm. The new specimen thus provides very close confirmation of the previous measurement as regards order of magnitude. It also suggests that chromosome length is unaffected by the early stages of pairing, the shrinkage leading to supercontraction being of later onset. This suggestion led to the intensive study of early pachytene which is the main concern of this paper.

MEASUREMENT AT LEPTOTENE IN A SECTION

Before proceeding to the main pachytene data it will be convenient to refer to one remarkable sporangium in which a measurement of an unpaired chromosome has been made at a stage which appears to be leptotene. The sporangium in question is shown in Pl. I, Fig. 1, and details of it in Pl. I, Figs. 2-4. The plant was a diploid and fixation quality excellent. The nuclei are strongly synizetic and the stage, in all except one of the mother cells, is early polarized pachytene, as may perhaps be judged from the detailed view of one end of a paired chromosome given in Pl. I, Fig. 3, which is an enlargement of part of the left-hand cell of Fig. 2. The right-hand cell of Pl. I, Fig. 2, which is also marked by an arrow in Fig. 1, differs from all the others in having no trace of pairing in its nucleus. It appears to have remained in the leptotene condition and one complete unpaired chromosome lies free in the synizetic fluid. This chromosome does not lend itself to complete demonstration by photography but parts of it are visible in Pl. I, Figs. 3 and 4, and a drawing is given in Text-fig. 2.

Such isolated cells at stages differing from their neighbours are unusual though not unknown in *Osmunda*. A suggestion of abnormality of some kind cannot be excluded, though such abnormality is as likely to be metabolic as morphological and would not necessarily invalidate the measurement. While any element of doubt is naturally to be regretted the specimen remains the only example of complete unpaired chromosome which I have ever obtained in a section and it is therefore the nearest approach yet available to a direct confirmation of the acetocarmine leptotene measurement, for which no suspicion of abnormality (other than that of technical origin) had been raised. Fortunately the new specimen is completely in harmony, both with the relative sizes shown by the acetocarmine series of measurements (see p. 174 below), and with the absolute sizes of all the observations on early pachytene to be dealt with next. Its length is 164 mm. at the magnification of the drawing or 41 μ in actual size.

¹ Dimensions expressed as mm. at a magnification of 1,000 are numerically equivalent to the actual size in microns, the latter units are, however, not used for acetocarmine measurements to avoid confusion with the real size.

OBSERVATIONS ON 'EARLY' PACHYTENE

The observations carried out on 'early' pachytene are contained in Text-figs. 3-43 and in Tables I-IV. Had there been any sign of significant difference between the measurements from polarized and from spread pachytene attention would have been concentrated on whichever of the two showed the greater



TEXT-FIGS. 2-5. Diploid *Osmunda*, various whole chromosomes from one preparation, of sectioned material. ($\times 4,000$.) Fixation No. 652 = 'outdoor plant, 7 p.m. May 5, 1939, warm and after rain' (optimum conditions). Text-fig. 2. Leptotene, see Pl. I, Figs. 1, 2, and 4 and description in text. Text-figs. 3-4. Polarized pachytene (see Table I and Pl. I, Fig. 5). Text-fig. 5. Spread pachytene (see Table I and Pl. I, Fig. 13).

state of chromosome elongation. No such difference was, however, detectable and the two stages, though separately listed, are treated as equivalent when the data are summarized. As was to be expected, the difficulty of obtaining whole chromosomes is very much affected by the number of homologues involved. The goal of ten pairs was achieved more than twice over for the diploid; for the triploid the two stages must be added together before ten trivalents are represented, while for the tetraploid it was not possible to find ten quadrivalents in one season's fixing, and supplementary observations were made in the following year. Since the latter proved to be somewhat abnormal they will be dealt with separately. Except for these, all the fixings used date from 1939.

The measurements in the tables are all quoted in mm. at a magnification of 4,000 in order to facilitate comparisons with the text-figures which are reproduced, as drawn, at this magnification. The confirmatory photographs in the

plate are either half or a quarter this size.¹ The dimensions in mm. at 4,000 diameters may be reduced to microns by dividing by four.

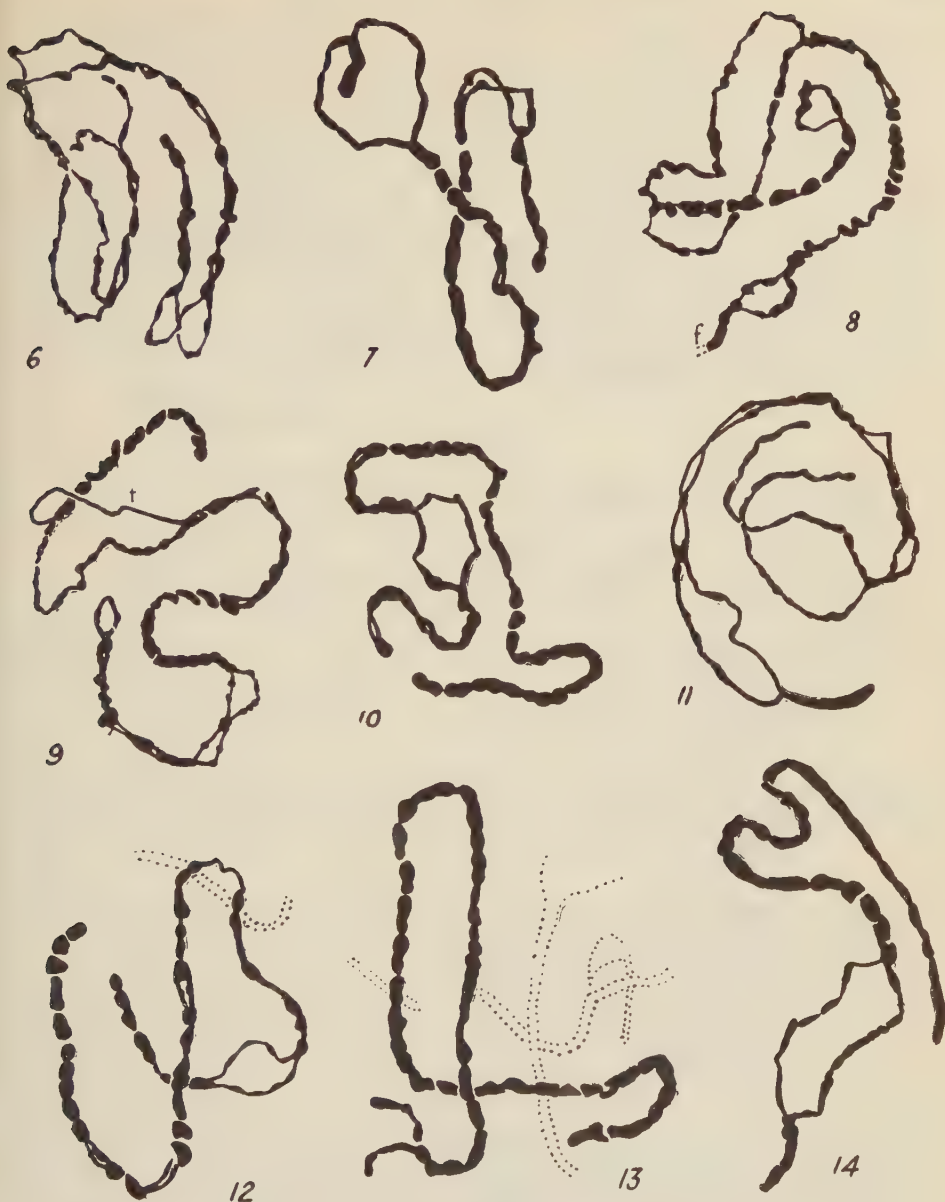
In listing the data it will be noticed that every chromosome has been given an index number (in the second column of each table) composed of two parts, a larger figure which is given first and which denotes the number of the fixing and a smaller figure, sometimes replaced by a letter of the alphabet, which is the individual number of the chromosome in that fixing. Further details regarding the fixation numbers are given in the legends to the text-figures and the object of including this information in the tables is to show to what extent uniformity or diversity of technical treatment may have affected the observations. The order in which the chromosomes are quoted is that of the text-figures.

TABLE I
Measurements of Chromosome Length in diploid Osmunda, 1939

Stage.	Fig. No.	Index No.	Length (mm. at $\times 4,000$).	Mean.	Length unpaired.	Mean.
Leptotene	Text-fig. 2; Pl. I, Figs. 1, 2, 4	652.A	164			
Polarized pachytene	Text-fig. 3; Pl. I, Fig. 5	652.B	168	161 mm. = 40 μ	55	44 mm. = 27%
	Text-fig. 4	652.C	175		55	
Spread pachytene	Text-fig. 5; Pl. I, Fig. 13	652.6	187		35	
	Text-fig. 6; Pl. I, Fig. 11	652.1	162		72	
	Text-fig. 7; Pl. I, Fig. 8	652.2	146		40	
	Text-fig. 8; Pl. I, Fig. 12	652.3	150		62	
	Text-fig. 9	652.4	168		60	
	Text-fig. 10	652.5	150		18	
	Text-fig. 11	652.10	150		80	
	Text-fig. 12	652.7	155		14	
	Text-fig. 13	652.8	166		10	
	Text-fig. 14	652.9	148		30	
	Text-fig. 46; Pl. I, Fig. 14	658.3	166		15	
	—	656.1	185		65	
	—	656.3	180		17	
	—	656.5	154		85	
	—	656.6	160		26	
	—	656.7	165		22	
	—	656.8	160		50	
	—	656.9	165		16	
	—	656.10	162		50	
	—	656.12	152		36	
	—	656.14	172		14	

With regard to the *diploid* data (Text-figs. 3-14 and Table I), little further

¹ But see footnote on p. 176.



TEXT-FIGS. 6-14. Diploid *Osmunda*, whole chromosomes at spread pachytene from the same preparation as Text-figs. 2-5. ($\times 4,000$.) Authenticating photographs of Text-figs. 6, 7, and 8 in Pl. I, figs. 11, 8, and 12; measurements in Table I.

comment is necessary except to explain that the ten chromosomes for which no text-figures are included were a preliminary series done without the aid of actual photography but using the photographic apparatus as described on p. 157. They were all found on one slide, which, though good, was less perfect

technically than some others obtained later. This series is of value in indicating the degree of difference of result which may legitimately be regarded as due to experimental error. In contrast with it, all the other diploid measurements were made with the aid of actual photographs. Except for chromosome 658.3 contained in Text-figure 46, all are from one slide, namely that which gave the measured leptotene described above. This preparation was of unusually good quality and was the last to be worked in detail. This series was therefore done with all the refinements that experience had shown to be helpful and is therefore probably the most accurate in the whole work. Confirmatory photographs are grouped in the right-hand bottom corner of Pl. I in which Figs. 11-14 relate to Text-figs. 6, 8, 5, and 46 respectively while the cells of Text-figures 3 and 7 appear also in Pl. I, Figs. 5, 6, and 8.

TABLE II

Measurements of Chromosome Length in Autotriploid Osmunda, 1939

Stage.	Fig. No.	Index No.	Length (mm. at $\times 4,000$).	Mean.	Changes partner.	Length incompletely paired.	Mean.
Polarized pachytene	Text-fig. 15; Pl. I, Fig. 16	643. 7	146 cut?		c. 11	?	
	Text-fig. 16	643. 1	148 + f		7	4	
	Text-fig. 17	643. 12	154		?	6	
	Text-fig. 18; Pl. I, Fig. 17	643. 5	174		7	10	
Spread pachytene	Text-fig. 19; Pl. I, Fig. 19	643. 3	140		5	50	
	Text-fig. 20; Pl. I, Fig. 20	643. 2	152		c. 5	10	
	Text-fig. 21	643. 14	170	159 mm.	5	10	16 mm. = 10%
	Text-fig. 22	643. 15	150		7	8	
	Text-fig. 23	643. 10	145		5	15	
	Text-fig. 24	703. 1	162		7	30	
	Text-fig. 25	703. 3	156		8	16	
	Text-fig. 26	703. 2	170		8	20	
	Text-fig. 44; Pl. I, Fig. 18	639. 1	180		7	15	

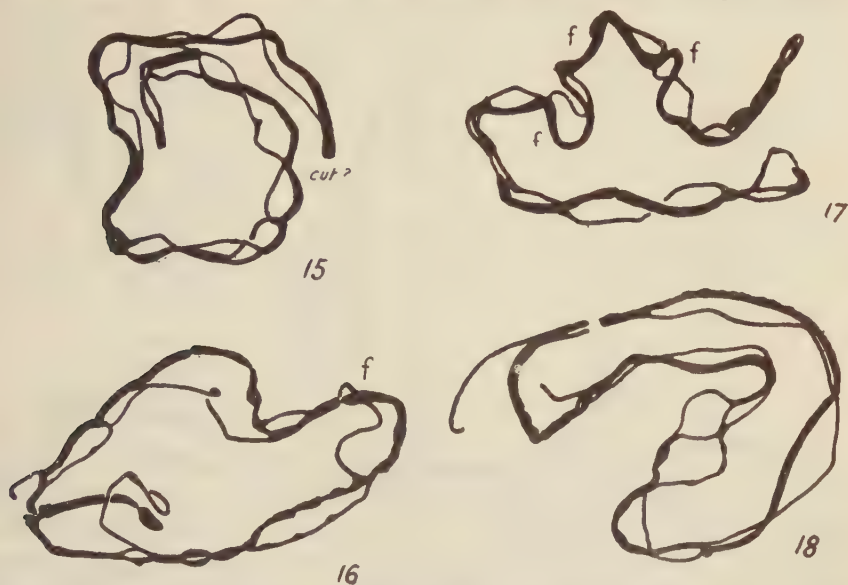
TABLE III

Measurements of Chromosome Length in Autotetraploid Osmunda, 1939

Stage.	Fig. No.	Index No.	Length (mm. at $\times 4,000$).	Mean.	Changes partner.	Length unpaired.	Mean.
Spread pachytene	Text-fig. 27	665. 1	145		5-7	55/2	
	Text-fig. 28	665. 2	174		4	22/2	
	Text-fig. 29	V. 2	155	155 mm.	0	0	13.5 mm. = 9%
	Text-fig. 30	V. 3	152		1	0	
	Text-fig. 31	V. 4	140		7	35/2	
	Text-fig. 32	V. 6	164		c. 7	50/2	

The *triploid* data (Text-figs. 19–26 and Table II) are self-explanatory except perhaps with regard to the significance of the figures representing unpaired length. These will be further discussed in the next section.

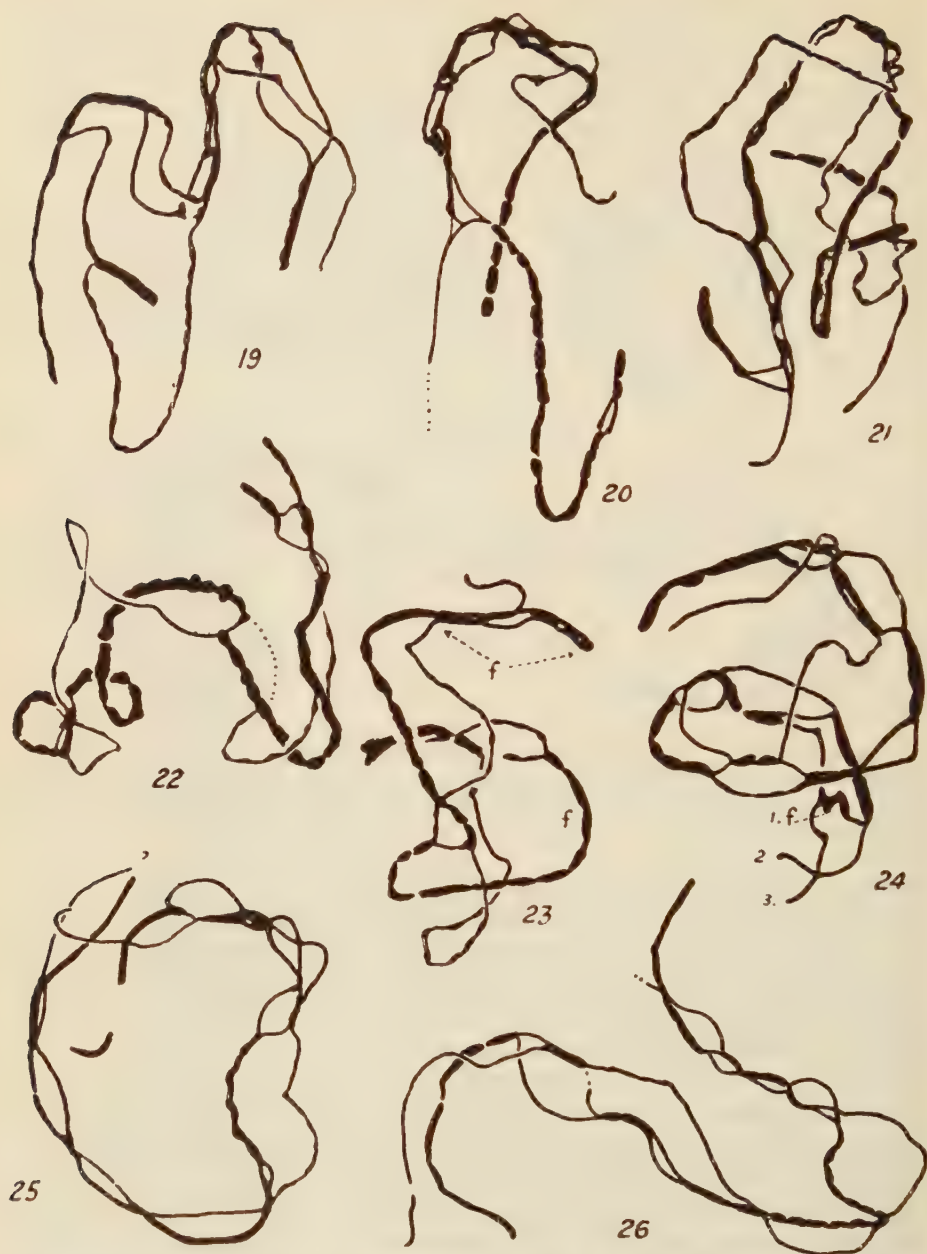
The *tetraploid* data of Table III (Text-figs. 27–32) are strictly comparable with the preceding except that the greater difficulty of making observations



TEXT-FIGS. 15–18. Autotriploid *Osmunda* (plant III), trivalent chromosomes at polarized pachytene ($\times 4,000$), from different preparations of the same fixing No. 643 = outdoor plant, May 4, 1939. The letter *f* denotes local foreshortening. Authenticating photographs of Text-figs. 15 and 18 in Pl. I, Figs. 16 and 17; measurements in Table II.

in quantity necessitated the use of a greater variety of plants. Plant V (Text-figs. 29–32) is a first-generation autotetraploid obtained by the self-fertilization of an aposporously produced diploid prothallus, but Text-figs. 27 and 28 were derived from a spore descendant of this plant and are therefore one generation removed from the original autopolyploid condition; no difference owing to this cause was, however, detectable.

The tetraploid data of 1940 contained in Table IV and Text-figs. 33–43 differed from the preceding in several respects. The fixings had been made rather late in the season on second-crop fertile fronds emerging in July. This had partly been necessitated by the unusually severe winter of 1939–40 which had killed many of the first-crop fertile leaves in the bud. The fixings were of excellent quality, as can be seen from Pl. I, Figs. 22–4, but they at once attracted attention by the fact that, contrary to previous experience of the polyploid plants, ten complete quadrivalents were obtained without difficulty on one slide. These chromosomes, however, were slightly shorter than usual, showed fewer changes of partner, and had very greatly reduced pairing length. A reduced chiasma frequency and other abnormalities to be



TEXT-FIGS. 19-26. Autotriploid *Osmunda*, trivalents at spread pachytene. ($\times 4,000$.) Text-figs. 19-23 from the same fixing as text-figs. 15-18; text-figs. 24-6 from fixing No. 703 = indoor plant (plant II), second-crop frond, June 1939. The letter *f* denotes local foreshortening. Authenticating photographs of Text-figs. 19 and 20 in Pl. I, Figs. 19 and 20; measurements in Table II.

TABLE IV

Measurements of Chromosome Length in Autotetraploid Osmunda, 1940

Stage.	Fig. No.	Index No.	Length (mm. at $\times 4,000$).	Mean.	Changes partner.	Length unpaired.	Mean.
Polarized pachytene	Text-fig. 33; Pl. I, Fig. 23	799. 11	140+f		c. 5	140/2	
	Text-fig. 34	799. 7	145		8	220/2	
Spread pachytene	Text-fig. 35; Pl. I, Fig. 25	799. 8	130		4	180/2	
	Text-fig. 36; Pl. I, Fig. 26	799. 12	145		3	210/2	
	Text-fig. 37	799. 5	150	148 mm.	4	120/2	58 mm. = 45%
	Text-fig. 38	799. 4	155		1	35	
	Text-fig. 39	799. 9	150		0	0	
		799. 10	155		0	45	
	Text-fig. 40	799. 2	156		0	25	
	Text-fig. 41	799. 3	152		0	20	
	Text-fig. 42	799. 6	156		2	120/2	
	Text-fig. 43	799. 1	145+f		3	150/2	

described later were subsequently discovered and on referring back to the living plant it was found that the belated fronds had failed to expand normally; though perfect morphologically, they had remained with diminutive pinnae and a total stature of about 6 in. All this suggested starvation effects of some kind due to temporarily adverse growing conditions. The results will therefore not be used to complete the tetraploid data as was at first hoped, but they are included here for use in the next section, and for other comparative purposes.

COMPARISON OF PACHYTENE PAIRING IN THE POLYPLOID SERIES

Surveying all the information contained in Text-figs. 2-32 and Tables I-III, it is clear, in the first place, that all the observations of total length are of the same general order. The means of the various sets of measurements (fifth column in all the tables), as well as the isolated measurement of leptotene (Table I), all fall between 155 mm. and 165 mm. (at a magnification of 4,000 diameters). This degree of difference is likely to be due to experimental error and is not excessive in view of the relative crudity of even the most skilful means of observing. Since the mean of the whole series coincides almost exactly with that of the most accurate set of observations (fixing 652, Table I), it may be stated in round numbers that the average length of a chromosome at its fullest extension in *Osmunda regalis* is of the order of 160 mm. at the magnification used, or 40μ .

The range of difference among the individual measurements from which the means are compiled is larger than the variation between the means themselves, and this is not entirely due to experimental error. It is known (Manton and Smiles, 1943) that genuine differences of length between individual chromo-

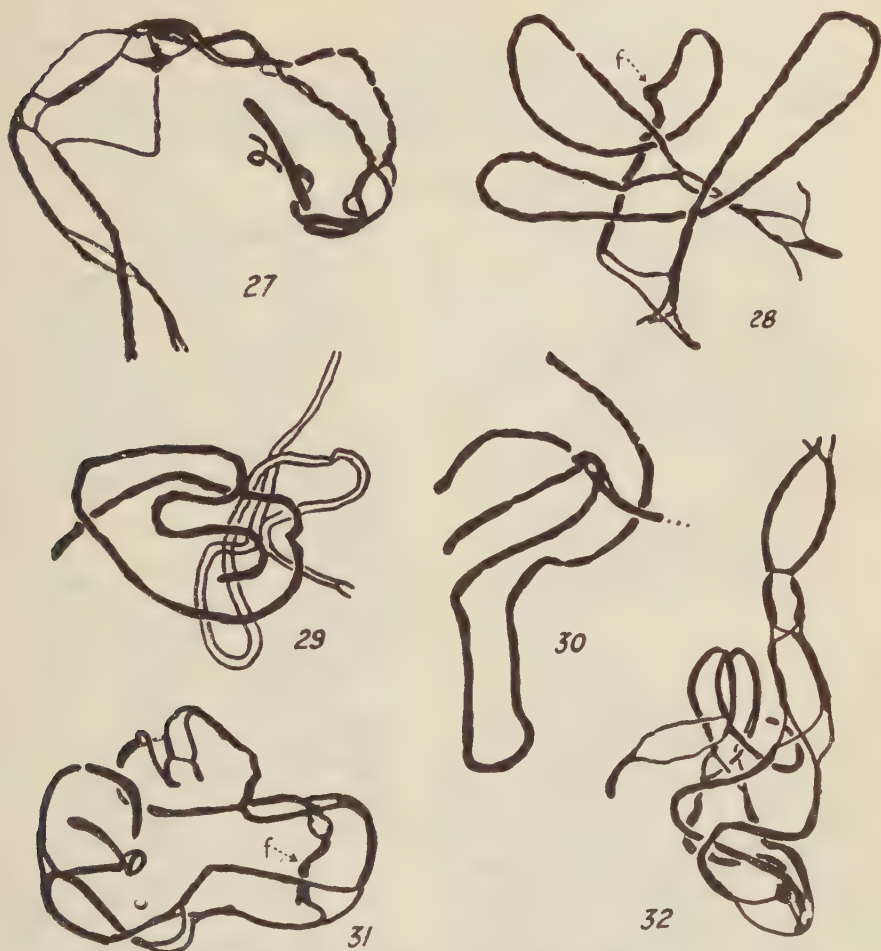
somes are of the order of 25 per cent. of the mean total length. Thus at a somatic division, where 16 coils per chromosome is the commonest number, a range from 14 to 18 coils was found among the different chromosomes in one cell. The range from 140 mm. to 187 mm. obtained at pachytene is clearly of the same order and the results thus agree very closely with previous knowledge.

The observations on relative pairing-length deserve further comment. A glance at the text-figures will show that, in every case, pairing is somewhat incomplete in spite of the exact homology of the pairing partners. This has long been known for diploid *Osmunda* and was correctly figured as long ago as 1910 by Grégoire. Unpaired regions may be placed along the length of a chromosome or at one end, but both ends are never thus affected. In the unpaired regions the homologues lie widely apart and the length of such places for every measured chromosome is given on the right-hand sides of the tables. In order to make the results comparable, however, it should be noted that for the triploid (Table II) the measurements do not include unpaired regions for which no partner exists (a length equivalent to that of the third chromosome in each trivalent), but they refer only to places where pairing could have taken place but has not done so, that is, to places where all three homologues are unpaired. For the tetraploid, where a quadrivalent is virtually equivalent to two pairs, the unpaired regions for each pair are measured separately, the results are then added together and the sum divided by two in order to obtain a value for the quadrivalent as a whole; this is the reason for expressing the entries in the sixth column of Tables III and IV as a fraction with 2 in the denominator.

Before making comparisons it is, further, convenient to reduce the actual measurements to a mean value for each group of chromosomes, as shown in the last column of each table, and to express this mean as a percentage of the mean total length for the group concerned. Expressed in this way, the two sets of diploid measurements work out as 23 per cent. and 27 per cent. unpaired, respectively, or as 25 per cent. in round figures. In contrast with this the triploid has 10 per cent. and the tetraploid 9 per cent. unpaired, the latter figure being less significant than the former owing to the smaller number of measurements. This deficiency, however, only hampers detailed comparison between tetraploid and triploid. In comparison with the diploid there is no doubt that in both polyploids the relative pairing-length has gone up.

This result is perhaps surprising in view of the fact that both polyploids possess a factor, which is absent from the diploid, tending to reduce pairing. This is the almost invariable occurrence of some failure of pairing at the site of a change of partner among the homologues. Failure of pairing due to this cause is visible in all the relevant text-figures and, particularly in the case of the tetraploid, it dominates the scene. Where changes of partner are few or absent (chromosomes V. 2 and V. 3, Table III) pairing may be virtually complete. In spite of the scantiness of the observations it is therefore probable that, in the tetraploid, the relative pairing-length is as high as it can be made

in this species and any further rise in chromosome numbers, should it occur, would not be expected to lead to any further increase in relative pairing unless the average numbers of changes of partner per multivalent should be reduced.



TEXT-FIGS. 27-32. Autotetraploid *Osmunda*, quadrivalents or pairs at spread pachytene. ($\times 4,000$.) Text-figs. 27-8 from fixing No. 665 = indoor plant (plant V. 7), second-crop frond, May 22, 1939; Text-figs. 29-32 from a second-crop frond of the indoor plant V fixed later in the summer of 1939. (Note: plant V is a first-generation autopolyploid and the parent of plant V. 7.) For measurements see Table III. The letter *f* denotes local foreshortening.

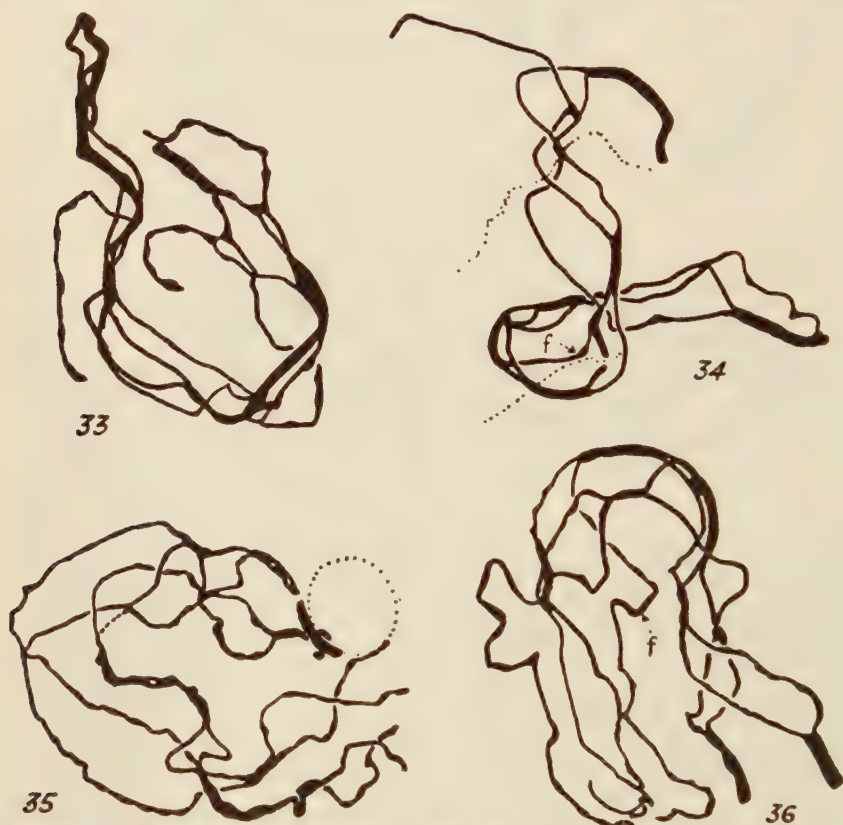
This, however, would not be an expected consequence of increased polyploidy.

The abnormal tetraploid material of 1940 (Table IV) showed fewer changes of partner and a marked reduction of pairing length, unpaired regions amounting to 45 per cent. of the whole. It may be suspected that one factor involved in this was a reduction in the ease and extent of chromosome movement since

their state of mutual entanglement was also less (cf. p. 159). Beyond the generalized nutritional cause already suggested the reasons for these differences are unknown, but some additional facts about this material will be found on p. 172.

SUPPLEMENTARY OBSERVATIONS ON SHRINKAGE AT 'LATE' PACHYTENE

In contrast to the various stages of 'early' pachytene just considered, 'late' pachytene has only been re-examined incidentally, to the extent required for



TEXT-FIGS. 33-6. Abnormal tetraploid material. Chromosomes from one slide. ($\times 4,000$.) Fixation No. 799 = indoor plant (plant IV), stunted second-crop frond July 16, 1940. Text-figs. 33-4. Polarized pachytene. Text-figs. 35-6. Spread pachytene. Authenticating photographs of Text-figs. 33, 35, and 36 in Pl. I, Figs. 22, 23, 25, 26; measurements in Table IV. The letter *f* denotes local foreshortening.

the seriation of the stages and to confirm the reality of the contraction, leading to supercontraction, previously reported (Manton, 1939). Thus Pl. I, Fig. 9, recalls the sporangium of Figs. 16 and 35 of the previous work in which a chromosome measurement of $18\frac{1}{2}$ microns was made. Two chromosomes from the sporangium of Pl. I, Fig. 9, are drawn in Text-figs. 47-8 and a photo-



TEXT-FIGS. 37-43. Abnormal tetraploid material, quadrivalents or pairs at spread pachytene from the same slide as Text-figs. 33-6. ($\times 4,000$.) Authenticating photograph of Text-fig. 38 in Pl. I, Fig. 24; measurements in Table IV. The letter *f* indicates local foreshortening.

graph of one of them is in Pl. I, Fig. 10. At the magnification of the drawings the measurements are 94 μ m. and 92 μ m. respectively or approximately 23 μ .

Similar demonstration of shrinkage in the triploid is contained in Text-fig. 45 and Pl. I, Fig. 21. This trivalent is closely comparable in attitude with the measured pair in the previous paper (*loc. cit.*, fig. 35), and it is approximately 80 μ m. long at the size of the text-figure, or 20 μ . Ocular demonstration of the shrinkage is easily obtained by casual comparison of this chromosome with the other text-figures and photographs published immediately beside it. Thus Text-fig. 44 and Pl. I, Fig. 18, show a chromosome from spread pachytene found on the same slide as the contracting pachytene under discussion; it is in a very similar attitude and manifestly about twice the length. Pl. I, Fig. 20, affords another easy comparison; it relates to the chromosome of Text-fig. 20.

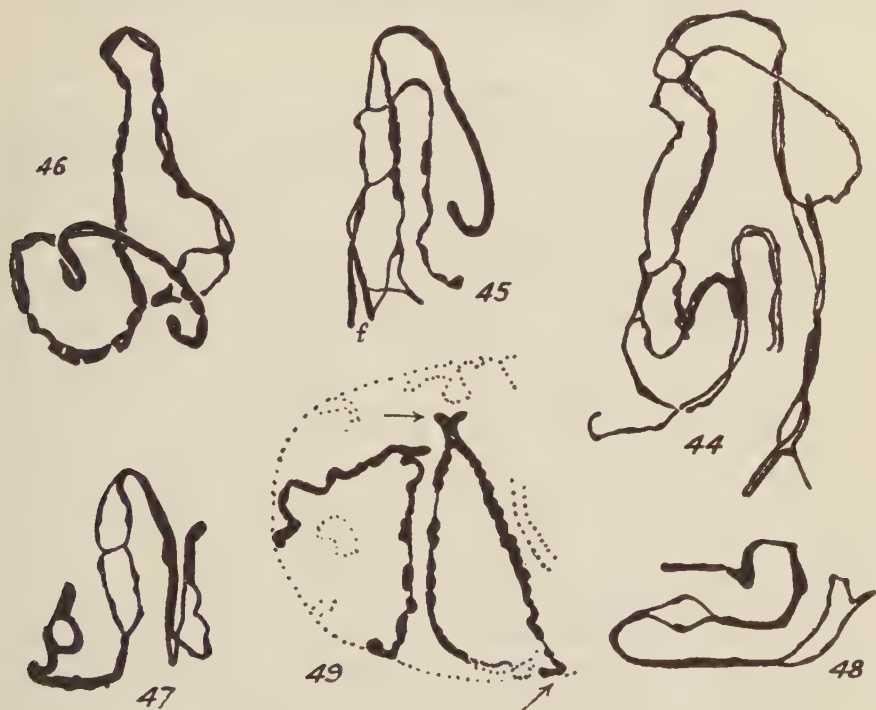
SUPPLEMENTARY OBSERVATIONS ON THE SUPERCONTRACTED LENGTH

The supercontracted state was studied fairly fully in Manton (1939) and only a few supplementary observations will be added here. The first of these refers to interkinesis, a stage not previously used for measurements. Pl. I, fig. 15, and Text-fig. 49 show an interkinetic nucleus at early prophase of the second meiotic division. The new spirals for that division have not visibly formed nor have the old ones been effaced in the majority of chromosomes. Across the centre of the nucleus some split V-shaped chromosomes are, however, suspended in such a way that the gyres of the relic coils have been straightened out and nearly the whole supercontracted length of one of them is contained in the plane of the photograph. It is of the order of 10 μ long, a figure which agrees closely with the order of size previously reported for the supercontracted condition. In contrast with this, the fully extended length at late pachytene under identical conditions may be seen in the immediately adjacent figure (Pl. I, Fig. 14, and Text-fig. 46). This chromosome was taken from the same slide as the interkinesis just referred to and is 41 μ long (see Table I).

Additional observations on the supercontracted state, of an unexpected kind, are contained in Pl. II. In the abnormal tetraploid material of 1940, not only was pairing depressed at pachytene but at the following metaphase, in many sporangia though not in all, the mother cells showed a very curious inability to retain the gyres of spiral structure in their chromosomes once these were congregated on the spindle. There was no detectable abnormality about diakinesis, and the normal compact form of the chromosomes characteristic of metaphase is retained in the cell of Pl. II, Fig. 36. In the two focal levels of the group of cells contained in Pl. II, Figs. 29, 30, on the other hand, all stages of de-spiralization can be seen, some of which are reproduced at a higher magnification in Pl. II, Figs. 31, 32. When fully de-spiralized the chromosomes appear as thin thread-like structures, and additional examples of the difference between this and their usual state are contained in Pl. II, Figs. 33-5. Pl. II, Fig. 34, is a portion of a normal meiotic metaphase from another

plant (plant V) in the previous year (1939); a double-rod shaped quadrivalent is marked by the arrow and a pair is visible to the right and a trivalent to the left of it. In contrast with these, the centre of Pl. II, Fig. 33, shows a despiralized quadrivalent of exactly the same form and arrangement of chiasmata as that of Pl. II, Fig. 34, at the same magnification, while parts of two despiralized pairs appear in Pl. II, Fig. 35.

While it cannot be certainly assumed that a change of length may not



TEXT-FIGS. 44-9. Whole chromosomes ($\times 4,000$), in various stages of elongation, contraction, and supercontraction. Text-fig. 44. Spread pachytene in triploid *Osmunda* (plant III), fixing No. 639 = outdoor plant, cool weather, April 29, 1939; authenticating photograph in Pl. I, Fig. 18, measurement in Table II. Text-fig. 45. Contracting pachytene from the same slide as the preceding; authenticating photograph in Pl. I, Fig. 21; description in text. Local foreshortening indicated by the letter *f*. Text-fig. 46. Spread pachytene in diploid *Osmunda*, fixing No. 658 = outdoor plant, May 8, 1939, another frond but same plant as fixing No. 652 used for Text-figs. 2-14. Authenticating photograph in Pl. I, fig. 14; measurement in Table I. Text-figs. 47-8. Two chromosomes from one sporangium at contracting pachytene from the same fixing as Text-figs. 2-14. Authenticating photographs in Pl. I, Figs. 9-10; description in text. Text-fig. 49. Interkinesis showing supercontracted length of split (V-shaped) chromosomes at early prophase of the second meiotic division, from the same slide as Text-fig. 46. Authenticating photograph in Pl. I, Fig. 15; description in text.

accompany despiralization, there is no detectable sign that it has done so to any considerable extent in cells such as those of Pl. II, Figs. 29-32, in which spiralized and despiralized chromosomes are present in the same nucleus. In all measured examples, the length of an individual chromosome is of the

order of 10μ and as an ocular demonstration of the supercontracted state these cells may therefore legitimately be used for comparison and contrast with the other significant measurements already described on Pl. I.

It may be said in passing that the association of de-spiralization with reduced or even complete elimination of pairing has been met with on other occasions in this species, notably in juvenile plants of varied genetical composition, when being fertile for the first time. This again suggests some nutritive deficiency, but whether the other two phenomena are causally connected or merely happen to occur together cannot yet be known.

CONCLUSIONS

The general conclusions to which this work has led can be very simply summarized. All the new measurements combine to show, beyond doubt, that the fully extended chromosome length is of the order of four times the supercontracted length. The fullest data are those presented here, but the previous measurements published in 1939 give the same result when once the doubt regarding the acetocarmine measurement is lifted. In sectioned material the comparison is between 40μ and 10μ . In acetocarmine the comparison was between 73 mm. for leptotene at a magnification of 1,000 and 18 mm. for the anaphase of the first meiotic division. In spite of the difference in absolute sizes the relative dimensions, in both techniques, are therefore as 4 : 1.

Secondly it has been shown that the fully extended state lasts for longer than was at first thought, i.e. from leptotene to spread pachytene, and is unaffected by the act of pairing itself. When contraction occurs, at the end of pachytene, the pairing process is already complete.

Pachytene pairing has been shown to differ in the polyploids in comparison with the diploid. Chromosome length is unaffected by polyploidy, but the relative pairing-length increases, in spite of the fact that, in the polyploids, change of partner among the homologous chromosomes invariably produces local failure of pairing at the site of the change. This observation is of some genetical interest, and it may be said in passing that a correlation has been established between changes of pairing length and changes of chiasma-frequency in all the types of plant studied. The data on chiasmata cannot however be included here from lack of space and will be published separately.

Lastly, it is profitable to collate the measurements for meiosis with those obtained on two previous occasions (Manton, 1939; Manton and Smiles, 1943) for mitosis. In the 1939 paper it was shown by measurement and calculation that there was no change in chromonema length, detectable by the methods used, between early prophase and anaphase, at a somatic division. All estimates of the somatic length worked out as of the order of 20μ when allowance was made for spiral structure. In Manton and Smiles, 1943, the greater accuracy attained in counting the number of coils at a mitotic metaphase confirmed this order of size by showing that the number of coils present in a prothallial mitosis was exactly twice that found in the same chromosome at the second

meiotic division, for which the diameter of coil was very similar. Since the supercontracted length is known to be of the order of 10μ , this again indicates 20μ as a correct estimate of the somatic length.

It may therefore be stated that, in round numbers, the lengths of a chromosome of *Osmunda regalis* L. in the supercontracted condition (metaphase and anaphase of both meiotic divisions), the mitotic condition (prophase to anaphase), and the fully extended condition (leptotene to early pachytene), are of the order of 10μ , 20μ , and 40μ respectively, a ratio which may be simply expressed as 1 : 2 : 4.

This simple expression is probably the most important fact which has so far been established in *Osmunda* and there are not yet available fully comparable figures for any other organism. The work of Sparrow, Huskins, and Wilson (1941) on *Trillium erectum* L. is the nearest approach, as far as the literature can be traced in war time, and it is of interest that they quote figures in a ratio of roughly 3 : 1 between leptotene and the supercontracted state. Unfortunately, their estimate of leptotene is not a direct measurement and until this is supplied it is not certain that the various assumptions which have to be made in obtaining such a figure indirectly will really bear the weight that has been put upon them.

The meaning of these differences in length cannot yet be fully known and many details must be added before the comparison between mitosis and meiosis, or between one organism and another, can be fully carried out. The state of rest and the conditions immediately bordering on it are, for example, completely unknown ground. Nevertheless the facts now ascertained bring the comparison previously made (Manton, 1939) between a chromosome and the fibrous proteins still nearer. In keratin, myosin, and collagen changes of length of exactly the same order as those now established for the chromosomes of *Osmunda* characterize the transition from one physical state to another. The various physical states differ from one another primarily by means of relatively simple, reversible, changes of molecular shape, and it is not unreasonable to suggest that a similar mechanism might operate in the case of the chromosomes. Whether such a mechanism would also, as suggested in 1939, provide the beginnings of a basis for understanding the very remarkable association which appears to exist between chromosome elongation and the phenomenon of pairing, must await further evidence. The possibility, however, adds interest to the inquiry which has here been summarized.

SUMMARY

1. A measurement of one complete chromosome at leptotene in a section has been made and a previous measurement in acetocarmine (Manton, 1939) has been confirmed.
2. Measurements of twenty odd chromosomes have been made in the early stages of pachytene in diploid *Osmunda*. They agree with the results for leptotene, and it is therefore concluded that chromosome length is constant during the early stages of pairing.

3. The existence of contraction, leading to supercontraction, in the later stages of pairing, has been confirmed.

4. Observations on early pachytene have been made on a smaller scale in triploid and tetraploid *Osmunda*, in the same year as those on the diploid. Chromosome length is similar in the polyploids but the relative completeness of pairing is increased.

5. Observations on some abnormal tetraploid material in a different year showed the simultaneous occurrence of greatly reduced pachytene pairing with a failure to retain the normal spiral structure at later stages. The cause and possible connexion of these two phenomena are uncertain.

6. Collating the new measurements of length with those provided in two previous papers (Manton, 1939; Manton and Smiles, 1943) a very simple relation has now been proved to exist between the various lengths characteristic of a chromosome at some of the more important stages of mitosis and meiosis. The fully extended length, the somatic length, and the supercontracted length are in a ratio of 4 : 2 : 1. This order of difference is exactly comparable to the changes of length accompanying changes of physical state in the fibrous proteins and a similarity of underlying mechanism is again suggested.

LITERATURE CITED

- GRÉGOIRE, V., 1910: Les Cinèses de Maturation dans les deux Règnes. *La Cellule*, xxvi. 223-422.
- LANG, W. H., 1924: On some Deviations from the Normal Morphology of the Shoot in *Osmunda regalis*. *Mem. and Proc. Manchester Lit. and Phil. Soc.*, xlviii. 53-67.
- MANTON, I., 1932: A Case of Induced Apospory in *Osmunda regalis*. *Journ. of Genet.*, xxv. 423-30.
- 1937: The Problem of *Biscutella laevigata* II. *Ann. Bot., Lond., N.S.*, i. 439-62.
- 1939: Evidence on Spiral Structure and Chromosome Pairing in *Osmunda regalis* L. *Phil. Trans. Roy. Soc. (London)*, B, ccxxx. 179-215.
- and SMILES, J., 1943: Observations on the Spiral Structure of Somatic Chromosomes in *Osmunda* with the Aid of Ultraviolet Light. *Ann. Bot., N.S.*, vii. 195-212.
- SPARROW, A. H., HUSKINS, C. L., and WILSON, G. B., 1941: Studies on the Chromosome Spiralization Cycle in *Trillium*. *Canadian Journ. of Research*, xix. 323-50.
- SPEAKMAN, J. H., 1931: The Micelle Structure of the Wool Fibre. *Proc. Roy. Soc. (London)*, A, cxxxii. 167-91.

DESCRIPTION OF PLATES I AND II

Illustrating Dr. I. Manton's paper on 'Chromosome Length in the Early Meiotic Prophases of *Osmunda*'. All figures from untouched microphotographs.

PLATE I¹

All preparations stained in haematoxylin after fixation in 2BD or 2BD diluted (see p. 156) and in most cases counterstained with bismarck brown.

Fig. 1. Diploid *Osmunda*. General view of the sporangium which gave the measured leptotene chromosome; for description see p. 160. ($\times 500$.)

Fig. 2. Two cells from the preceding at a different focal level. ($\times 1,000$.)

¹ In the process of reproduction this plate has been reduced by an amount which approximates to 9.3 per cent. of the original size. The magnifications listed should be emended accordingly and allowances made if accurate comparisons with plate and text-figures and with the tables are desired.

- Fig. 3. Detail of a piece of paired chromosome from the left-hand cell of Fig. 2. ($\times 3,000$.)
- Fig. 4. Detail of a piece of the unpaired chromosome from the right-hand cell of Fig. 2. ($\times 3,000$.) A drawing of the whole chromosome is given in Text-fig. 2, p. 161.
- Fig. 5. Diploid *Osmunda*. Polarized pachytene, the cell of 652 B (see Text-fig. 3 and Table I), from the same slide as Fig. 1. ($\times 1,000$.)
- Fig. 6. Diploid *Osmunda*. A sporangium in spread pachytene from the same preparation as Fig. 1. ($\times 500$.) A chromosome from this sporangium is in Fig. 11.
- Fig. 7. Diploid *Osmunda*. Spread pachytene from another preparation at a higher magnification. ($\times 1,000$.) A chromosome from this sporangium shown in Fig. 14.
- Fig. 8. Diploid *Osmunda*. Spread pachytene from the same preparation as Fig. 1. Chromosome 652.2 of Text-fig. 7. ($\times 1,000$.)
- Fig. 9. Diploid *Osmunda*. Contracting pachytene from another preparation. ($\times 1,000$.) A chromosome from this sporangium in Fig. 10 and in Text-figs. 47-8.
- Fig. 10. The chromosome from the preceding sporangium drawn in Text-fig. 47. ($\times 1,000$.)
- Fig. 11. Diploid *Osmunda*. Spread pachytene. Chromosome 652.1 of Text-fig. 6 and Table I. ($\times 2,000$.)
- Fig. 12. The same. Chromosome 652.3 of Text-fig. 8 and Table I. ($\times 2,000$.)
- Fig. 13. The same. Chromosome 652.6 of Text-fig. 5 and Table I. ($\times 2,000$.)
- Fig. 14. The same but another preparation (that of Fig. 7). Chromosome 658.3 of Text-fig. 46 and Table I. ($\times 2,000$.)
- Fig. 15. Interkinesis from the same slide as the preceding showing the supercontracted length (see Text-fig. 49 and text, p. 172). ($\times 2,000$.)
- Fig. 16. Triploid *Osmunda*. Polarized pachytene showing chromosome 643.7 of Text-fig. 15 and Table II. ($\times 1,000$.)
- Fig. 17. The same, showing a part of chromosome 643.5 of Text-fig. 18 and Table II. ($\times 2,000$.) (For convenience of space the photograph is turned at right angles relative to the drawing.)
- Fig. 18. Triploid *Osmunda*. Spread pachytene. Chromosome 639.1 of Text-fig. 44 and Table II. ($\times 2,000$.)
- Fig. 19. The same. Chromosome 643.3 of Text-fig. 19 and Table II. ($\times 2,000$.)
- Fig. 20. The same. Chromosome 643.2 of Text-fig. 20 and Table II. ($\times 2,000$.)
- Fig. 21. Triploid *Osmunda*. Contracting pachytene. The chromosome of Text-fig. 45 showing effect of shrinkage as in Fig. 10. The specimen closely comparable in attitude and length with the previously measured example of 'late' pachytene in Manton, 1939, fig. 35. ($\times 2,000$.)
- Fig. 22. Tetraploid *Osmunda* in the abnormal year of 1940 showing polarized pachytene of normal type though with reduced chromosome pairing. ($\times 1,000$.)
- Fig. 23. A part of a quadrivalent, number 799.11 of Text-fig. 33 and Table IV, from the sporangium of Fig. 22. ($\times 2,000$.)
- Fig. 24. Tetraploid *Osmunda*. Spread pachytene from the same slide as the preceding showing a part of chromosome 799.4 of Text-fig. 38 and Table IV. ($\times 1,000$.)
- Fig. 25. The same, showing part of chromosome 799.8 of Text-fig. 35 and Table IV. ($\times 2,000$.)
- Fig. 26. The same showing part of chromosome 799.12 of Text-fig. 36 and Table IV. ($\times 2,000$.) This is the only quadrivalent lending itself to photographic comparison of total length with the trivalent of Fig. 18, &c., and with various pairs elsewhere in the plate.

PLATE II

Figs. 27-8. The cell of Text-fig. 1 showing a trivalent at late zygotene or early polarized pachytene in a triploid plant (plant II). Acetocarmine squash made permanent by McClintock's method. (Fig. 27 $\times 1,000$; Fig. 28 $\times 2,000$.)

Figs. 29-30. Two focal levels through the same group of cells at metaphase of the first meiotic division in the abnormal tetraploid material of 1940 (plant IV; see p. 172) showing various stages of despiralization. ($\times 1,000$.) Section stained in haematoxylin, counterstained with bismarck brown.

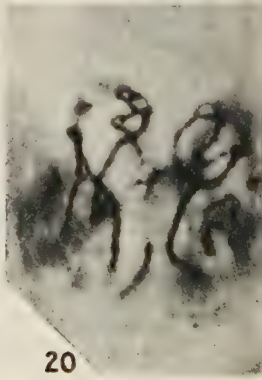
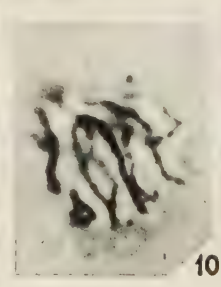
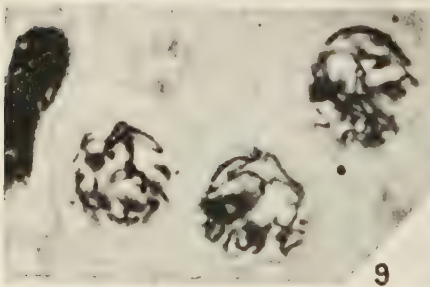
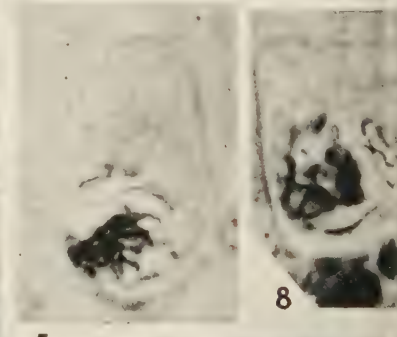
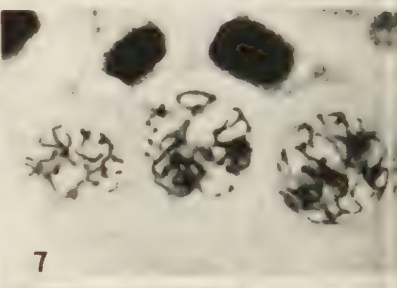
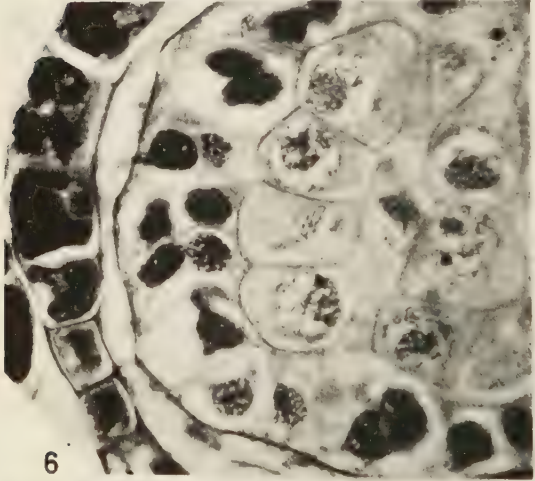
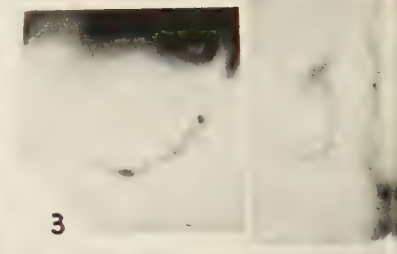
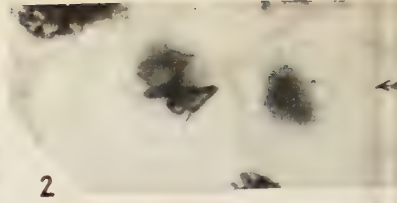
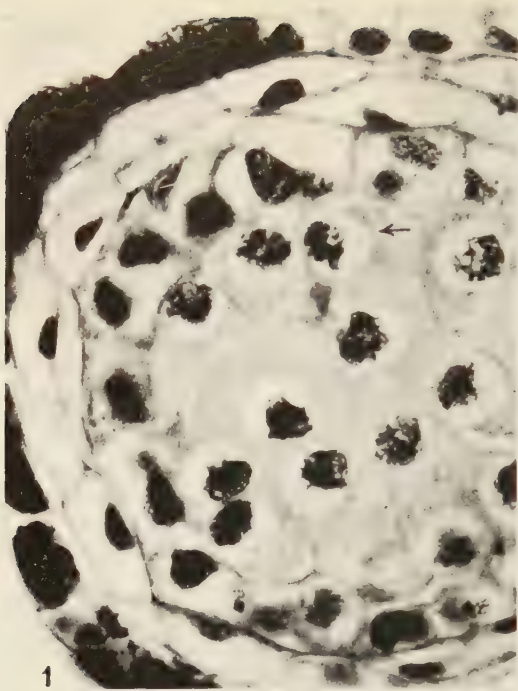
Figs. 31-2. Enlarged detail of two focal levels of the top right-hand cell of the preceding. ($\times 2,000$.)

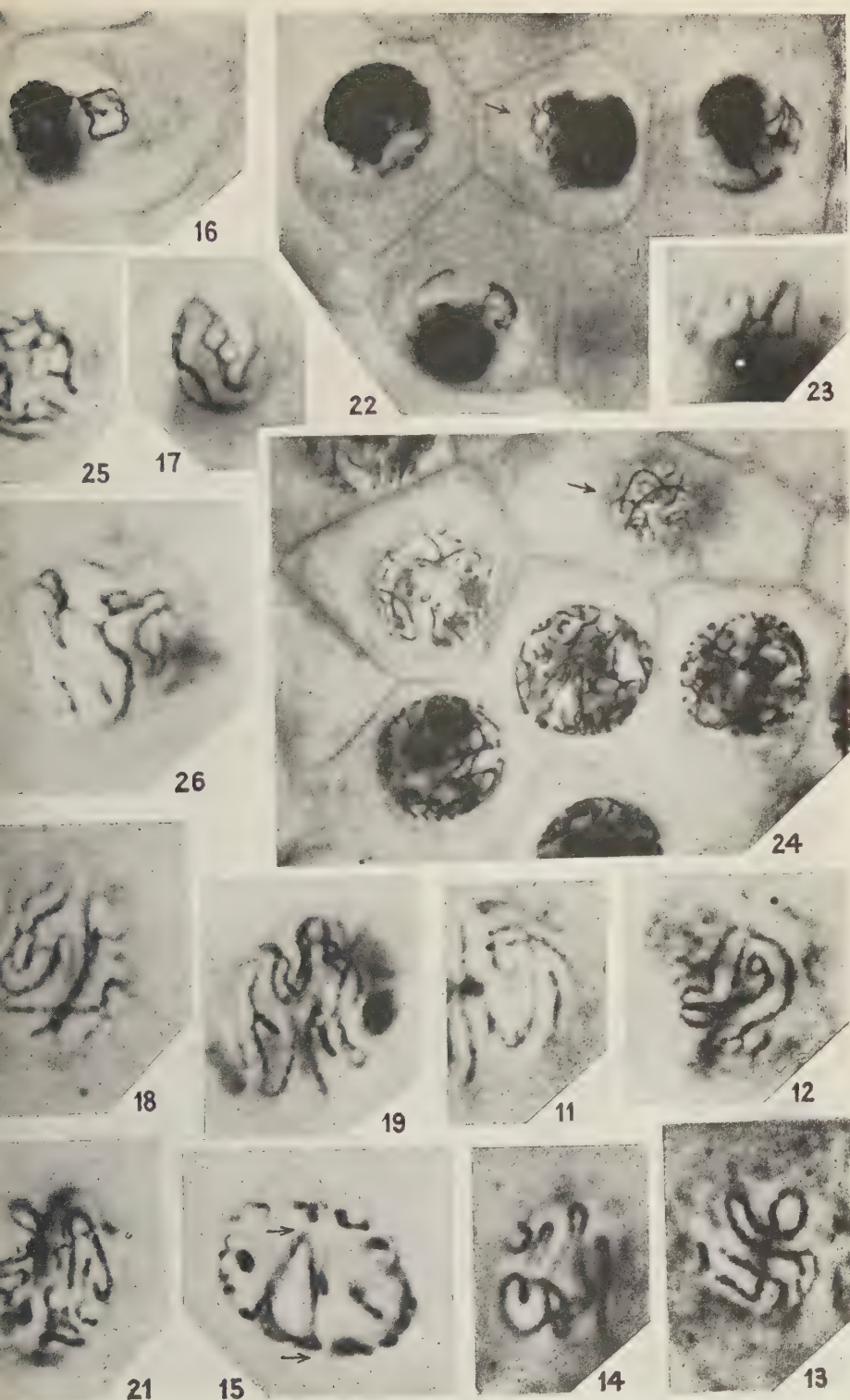
Fig. 33. Detail of a despiralized quadrivalent from the same material. ($\times 2,000$.)

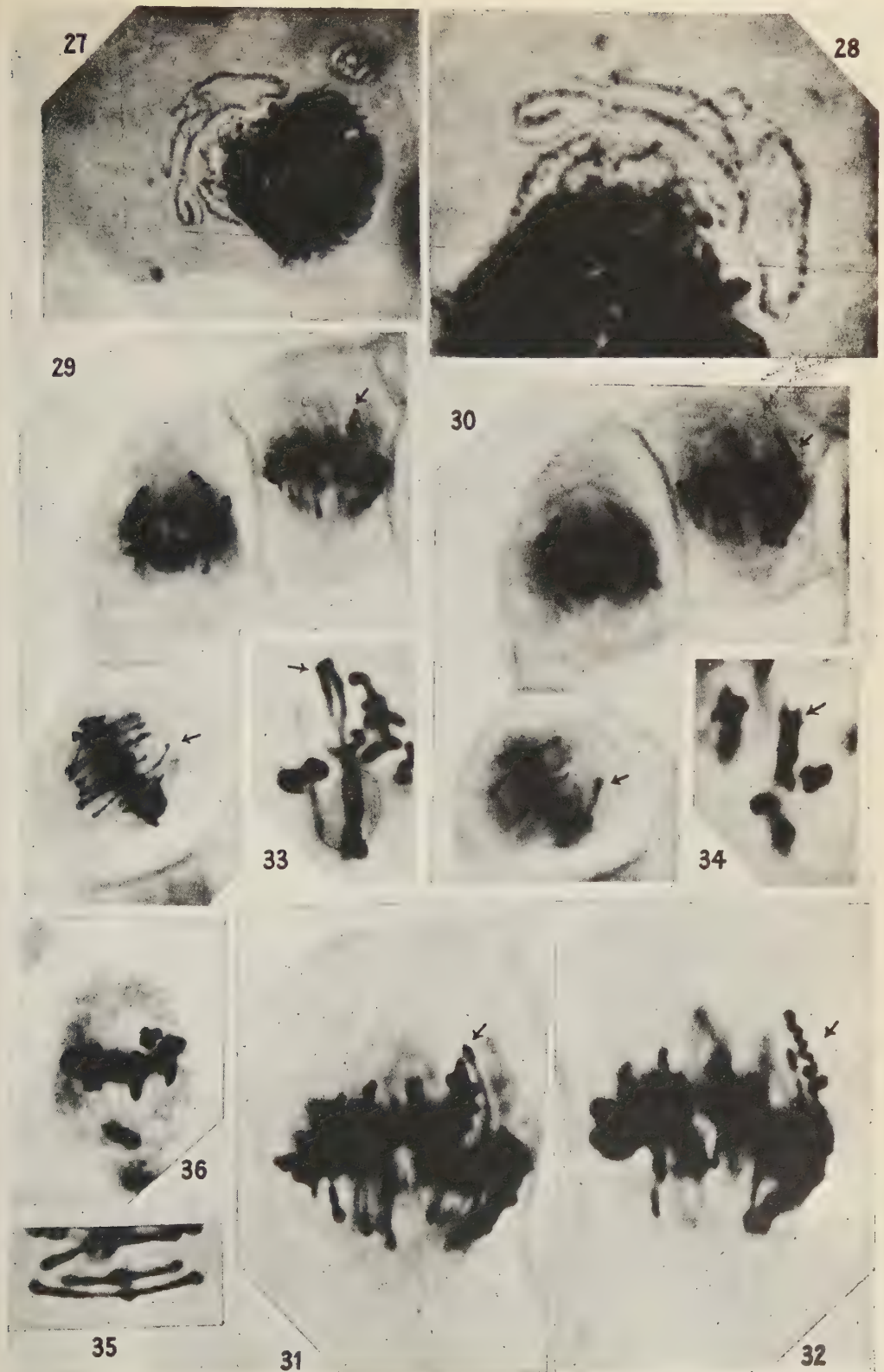
Fig. 34. Detail of a normal quadrivalent of comparable morphology to that of Fig. 33 for comparison with it. A pair is visible to the right of the quadrivalent and a trivalent to left of it. Fixing from a normal plant (plant V) in the preceding year (1939). ($\times 2,000$.)

Fig. 35. Parts of two pairs from the abnormal tetraploid material for comparison with the quadrivalent of Fig. 33; in each, a portion of the two chromosomes below the chiasma is out of view. ($\times 2,000$.)

Fig. 36. Relatively normal spindle and chromosomes from another sporangium at the first meiotic division in the abnormal tetraploid material of 1940, for comparison with Fig. 29 ($\times 1,000$.) For description see p. 172.







Homologies of the Embryo Sac of Angiosperms

BY

B. G. L. SWAMY

AND

M. J. THIRUMALACHAR

With one Figure in the Text

THE homology of the embryo sac in angiosperms has always been a subject of keen interest to workers in plant embryology. Views on this homology have recently been put forward by Porsch, by Schuroff, and by Thompson. Both Porsch and Schuroff derive the normal 8-nucleate embryo sac from archegonia of a typical gymnosperm, while Thompson advocates a Gnétalian origin. The unsatisfactory nature of Schuroff's and of Thompson's views has already been pointed out by Maheshwari (1937). According to Porsch, the normal 8-nucleate embryo sac of angiosperms consists of two archegonia, one at either pole. Many of the recent investigators have pointed out that the egg and the polar nucleus of the micropylar end are sister nuclei. Porsch's view is that the four nuclei at each end represent an archegonium. The synergids are equated with the neck cells of gymnosperms and the polar nucleus with the ventral canal nucleus.

If we accept Porsch's view, which seems a tenable one, we have to suppose that during the evolution of the embryo sac the micropylar archegonium alone remained functional forming a specialized egg apparatus, while the one at the chalazal end became nutritive in function either by the degeneration of the nuclei, thus contributing nutritive material, or by becoming persistent and haustorial.

Instances are not lacking where, up to a certain stage, the development is similar at both the micropylar and antipodal ends. In *Heptapleurum venulosum* Seem, for instance, the antipodals become cellular, developing the same characteristic vacuolations of the synergids as at the micropylar end; similar cases have also been reported in a large number of other plants. A survey of the literature shows a few instances of embryo-sac reversal recorded as abnormalities. Only in *Atamasgo texana*, an apogamous plant (Pace, 1913), were cases of embryo-sac reversals noticed in appreciable numbers. In *Leiphanimos* sp. and *Cotylanthers tenuis* (Oehler, 1927), which are reported to show the reversed polarity habitually, the problem becomes complicated as they are both saprophytic and the last one apogamic. According to Oehler 'the seed of the above two species are externally orthotropic but internally

they are anatropic, i.e. the embryo sac is oriented as in the seeds of anatropous ovules'.

Such instances, though sporadic, are of marked significance. They lend support to Porsch's view of the embryo sac in angiosperms as composed of two archegonia. We can visualize the hypothetical embryo sac with the two groups of four nuclei at either pole having equal chances of assuming the function of the sexual process. The cases of reversed polarity might be nothing but the suppression of the micropylar archegonia. In plants like *Atamasco texana* normal embryo sacs and those with reversed polarity are both common, their formation being perhaps determined by the degeneration of chalazal or micropylar archegonia. That no fertilization has been observed in embryo sacs with reversed polarity cannot be considered a serious objection to the view since the egg is situated so far away from the pollen tube at the micropyle.

The main objection to Porsch's view is put thus by Maheshwari (1937)—'what reason is there for the ventral canal nucleus (upper polar nucleus) to leave its position above the egg and come down to the centre of the embryo sac to fuse with another nucleus from the chalazal end which is also a ventral canal nucleus of the second archegonium?'

It may be recalled that the fusion of the polar nuclei is not a necessary act for the production of the endosperm. For instance in *Helosis guayanensis* (Chodat and Bernard, 1900) the polar nucleus from the chalazal end does not take part in the formation of endosperm; similarly in *Antennaria alpina* (Juel, 1898) the endosperm is formed without the fusion of the polars. On the other hand, in *Limnocharis emarginata* (Hall, 1902) no antipodal polar is formed and the endosperm is formed even before fertilization and independent of any fusion. From these examples Coulter and Chamberlain (1912) point out: 'In any event the formation of endosperm without antecedent fusion is clear enough in some cases and indicates that while fusion usually serves to stimulate growth and cell division, it is not an absolute prerequisite.'

The endosperm is primarily a nutritive tissue, whatever may be its cytological implications. Among gymnosperms its formation is a continuous process from the beginning of the female gametophyte and it is a gametophytic tissue. In the angiosperms, on the other hand, it is mostly of a 3n generation, and instances are not wanting where it is of the same cytological nature as in the gymnosperms.

From these considerations it is manifest that the nutritive tissue is built up (i) by the two polar nuclei either by direct division without preceding fusion, e.g. *Lemna minor* (Caldwell, 1899) or (ii) with preceding fusion which imparts the necessary stimulus for further divisions, e.g. *Monotropa uniflora* (Shibata, 1902), and (iii) by division only after the triple fusion which explains the incapacity to form the nutritive tissue in the absence of fusion with the second male nucleus. Many cases are on record where the male nuclei in addition to providing the stimulus, impresses the character of the male parent on the endosperm, the phenomenon of xenia.

The main objection to considering the polar nucleus as comparable with the

ventral canal nucleus is the migration of the polar nuclei to the centre and their consequent fusion, as also their fusion with the second male nucleus in the process of development of the nutritive tissue. The following suggestions may reduce the objections raised against acceptance of Porsch's view. (a) It is possible that all the various behaviours—division of the polar nuclei after triple fusion, division without their fusion, &c., and the migration of the polar nuclei (ventral canal nuclei) from their respective positions to the centre

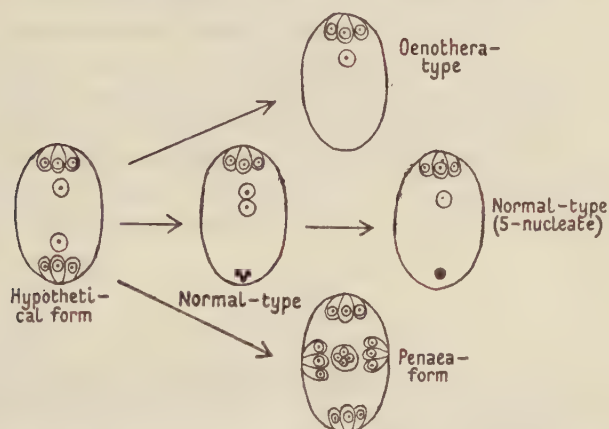


Diagram to show the probable derivation from the hypothetical type of some of the modern angiosperm types of embryo sac.

and subsequent fusion—may be only a secondary phenomenon to provide a stimulus for further divisions. (b) It is well known that the synergids secrete certain substances responsible for the attraction of the pollen tube towards the micropylar end. The migration of the polars to the centre and their mutual attraction and their universal position near to the egg apparatus before fertilization might also be due to some stimulus of similar nature. (c) In many species of *Ephedra* as *E. campylopoda* (Herzfeld, 1922), and in *Abies balsamea* (Hutchinson, 1915) cases of fusion of the second male nucleus with the ventral canal nucleus have been recorded. In *Ephedra* it is further surmised that the ventral canal nucleus, after this 'double fertilization', develops a small amount of nutritive tissue surrounding the fertilized egg. It is well known that in all gymnosperms, including *Ephedra*, the endosperm is formed before fertilization. The fusion of the ventral canal nucleus with the second male nucleus, which also might contribute some nutritive tissue by its further divisions in *Ephedra*, does not detract in any way from its status as a ventral canal nucleus. It is therefore manifest that in angiosperms each of the polar nuclei is homologous with a ventral canal nucleus and they are functional, producing a nutritive tissue. Their precedent fusion before double fertilization is only a secondary phenomenon to provide the necessary stimulus.

With Porsch's concept of there being two archegonia in the normal 8-nucleate embryo sac, which is reasonable and in keeping with facts, we can

presume the probable condition in some of the other types of embryo sacs in the angiosperms. The *Oenothera*-type of embryo sac, for instance, could be fittingly compared with a single archegonium. The two synergids, the egg and the polar, can be equated with the two neck cells, the egg and the ventral canal nucleus respectively. The same view could be extended to the *Penaea* type of embryo sac where four groups of four nuclei at each corner of the embryo sac are organized. Even though only the micropylar group of nuclei organize the egg apparatus, the primary endosperm nucleus is formed by four nuclei to which each of the four groups of nuclei contribute one. Moreover, all the four groups show a similar organization. One may consider that with four archegonia present the four nuclei of the primary endosperm are the ventral canal nuclei of the four archegonia, fusing in the centre to provide the necessary stimulus. These two instances are referred to not with the wish to fit all the types of embryo sac known among the angiosperms into Porsch's concept, but because these two cases so clearly illustrate Porsch's view.

LITERATURE CITED

- CALDWELL, O. W., 1899: On the Life-history of *Lemna minor*. Bot. Gaz., xxvii. 37.
 CHODAT, R. and BERNARD, C., 1900: Sur le sac embryonnaire de l'*Helosis guayanensis*, Journ. Bot., xiv. 72. (Original not seen.)
 COULTER, J. M. and CHAMBERLAIN, C. J., 1912: Morphology of Angiosperms. New York.
 GOPINATH, D. M., 1943: Embryological Studies on some Members of the Araliaceae. (In press.)
 HALL, J. G., 1902: An Embryological Study of *Limnocharis emarginata*. Bot. Gaz., xxxiii. 214.
 HERZFELD, S., 1922: *Ephedra campylopoda* Mey. Morphologie der weiblichen Blute und Befruchtungsvorgang. Denkschr. Akad. Wissen. Wien Math-Naturkl., xcvi. 243-68. (Original not seen.)
 HUTCHINSON, A. H., 1915: Fertilization in *Abies balsamea*. Bot. Gaz., lx. 457.
 JUEL, H. O., 1898: Parthenogenesis bei *Antennaria alpina* (1). R. Br. Bot. Centralbl., lxxiv. 369.
 MAHESHWARI, P., 1937: A Critical Review of the Types of Embryo-sacs in Angiosperms. New Phyt., xxxvi. 359.
 OEHLER, E., 1927: Entwicklungsgeschichtlich-cytologische Untersuchungen an einigen saprophytischen Gentianaceen. Planta, iii. 641. (Original not seen.)
 PACE, L., 1913: Apogamy in *Atamasco*. Bot. Gaz. lvi. 376.
 SHIBATA, K., 1902: Experimentelle Studien über die Entwicklung des Endosperms bei *Monotropa*. (Vorläufige Mitteilung.) Biol. Centrabl., xxii. 705.

Studies in the Vegetative Growth and Anatomy of the Tea Plant (*Camellia thea* Link.) with Special Reference to the Phloem

II. Further Analysis of Flushing Behaviour

BY

T. E. T. BOND

(*Tea Research Institute of Ceylon, St. Coombs, Talawakelle, Ceylon*)

With thirteen Figures in the Text

	PAGE
INTRODUCTION	183
DEFINITIONS	184
MATERIALS AND METHODS	184
APICAL ACTIVITY AND THE FLUSHING CYCLE	185
General statement	185
Mutual relationships	190
The production of scale and flush primordia	192
GROWTH AND 'DETERMINATION' OF THE PRIMORDIA	192
Applicability of the logistic curve	192
Appendages and internodes	195
The growth rates of successive primordia	197
The early phases of primordial growth	201
Primordial growth-rates and apical activity	205
VASCULARIZATION BELOW THE APICAL BUD	205
SUMMARY OF MUTUAL RELATIONSHIPS	207
DISCUSSION	210
SUMMARY	213
LITERATURE CITED	216

INTRODUCTION

IN the first paper of this series (Bond, 1942) the normal periodicity of growth of the vegetative shoots of tea under Ceylon up-country conditions was briefly described and attention was drawn in the discussion to the suitability of the tea plant for studies on the problems of apical growth and 'foliar determination'. The present communication is the result of further investigations undertaken from this point of view. The flushing behaviour of tea is here analysed in terms of the two component variables, *apical activity* involving the production and emergence at the apex of new primordia, and *primordial growth-rate* as affecting the subsequent development of the primordia into cataphylls or foliage leaves. These two factors are discussed in relation to each other and in relation to a third variable, namely, the hypothetical supply of water and nutrients as measured by the vascularization of the tissues within a given distance of the growing point. In this way a

consistent theory of the mechanism of flushing behaviour is built up which will form the basis of future anatomical and histological studies.

DEFINITIONS

In general, the terminology of the previous paper is adhered to. A slight modification is introduced in the designation of the stages of development of the flush shoot (Bond, 1942, p. 610) whereby stages E, F, F+, G, &c., are now based solely on the numbers of appendages expanded, irrespective of kind, beginning with the first scale leaf at stage E. Thus stage F+, for instance, describes any shoot with three expanded appendages; these may be either two scales and a fish leaf, three scales only, or two scales and the first flush (foliage) leaf. The term *flush period* (or *flushing cycle*) is applied both to the process and to the result of the growth period. Typically, it is counted from the exposure of the *banji* bud at stage A until the bud again 'goes *banji*' at stage A', which then denotes the beginning of the second cycle and is followed by stages B', C', and so on. Similarly, the appendages of the bud or of the shoot developed from it are numbered in the order of their development relative to the given flush period, the *first* appendage always being the outermost scale leaf. The last flush leaf of the previous cycle is occasionally referred to as appendage 'o'. The *appendage values* of the successive flush stages denote the *total* number of appendages produced by the bud from stage A onwards. Thus, the appendage value of a stage C' shoot will be made up of the full complement of the flush period already ended, together with the terminal *banji* bud.

Three measures of rate or activity will be given frequent mention and are to be carefully distinguished. *Flushing rate* is a measure of outward activity as evidenced by the swelling of the bud and the subsequent expansion of the scales and foliage leaves of the current flush period. *Apical activity*, on the other hand, refers to the emergence of new primordia at the growing point, a process revealed only by microscopic examination of the buds and not necessarily deducible from their external appearance. *Growth rate* is used in the mathematical sense as a measure of increase in length, usually of the primordia and their internodes. The values attached to these quantities, and the methods of computing them, will be apparent from the ensuing sections.

MATERIALS AND METHODS

The principal data were obtained from three lots of shoots, as follows:

1941-2. Thirty-seven out of the 70 shoots originally recorded from July 1941 to January 1942 (Bond, 1942, p. 608) completed at least a single cycle from stage A to A' inclusive. The records from these shoots were brought to a common starting-point as at stage A. Appendage values for the different stages were derived from dissections of buds taken from the same bushes in November 1941 and later, usually 10 buds of each stage being averaged.

1942-3 (a). Fifty stage A shoots were tagged on a single bush included among those from which the 1941-2 records were taken. The bushes had

been pruned about 8 months previously. Five shoots were sampled at a time over a period of 6 months from the beginning of December 1942 at regular 1-, 2-, and later 4-week intervals.

1942-3 (b). A parallel batch of fifty shoots, taken from three young unpruned bushes about 3 years old. These were rather darker leaved and of lower *jât* than the others. The two lots of shoots were sampled at the same times, the stage of development being recorded and the leaves and internodes measured individually.

All bud dissections were made under a Spencer binocular at a magnification of 11, measuring in micrometer units of 0.083 mm. Thus no primordium smaller than this was recognized in assessing appendage values. Macroscopic measurements were made to the nearest millimetre and, as far as possible, all calculations were carried through in unconverted units according to the nature of the measurements.

APICAL ACTIVITY AND THE FLUSHING CYCLE

General statement.

Bearing in mind that the number of appendages in the apical bud can be determined only by dissection which involves the use of sampling, there are three ways in which the course of apical activity can be determined. These are discussed below for the 1941-2, the 1942-3, and the combined data.

1941-2 shoots. Average appendage values (*total* appendages) and flush values (*expanded* appendages), as computed from the proportions of the different flush stages, were plotted against weekly intervals of time from the common starting-point at stage A. Appendage values for the first cycle were those derived from independent samples by dissection, as shown in Table I. The values for the second cycle were calculated individually according to the total for the first cycle in the shoots concerned. The average flush cycle for the 37 shoots consisted of 6.7 expanded appendages and lasted 14.1 weeks from stage A to A'. The *average* appendage value for stage A' is, therefore, $6.7 + 3.5 = 10.2$ appendages.

TABLE I

Appendage Values, 1941-2

Stage.	Total appendages.
A	3.5
B	4.0
C	4.7
D	6.5
E	7.3 (1)
F	9.0 (2)
F+	9.0 (3)
G	9.9 (4)
G+	10.0 (5)
G++	10.3 (6)

Figures in brackets relate to *expanded* appendages.

The two graphs for average appendages (y), total and expanded, against time (x) are shown in Fig. 1. Up to 9 weeks the points are based on the full 37 shoots; thereafter the number of shoots available declines until from week 19 onwards it is less than 25.

The method of presentation introduces a considerable 'smoothing' effect. Flushing is essentially discontinuous in nature, ceasing abruptly for the time

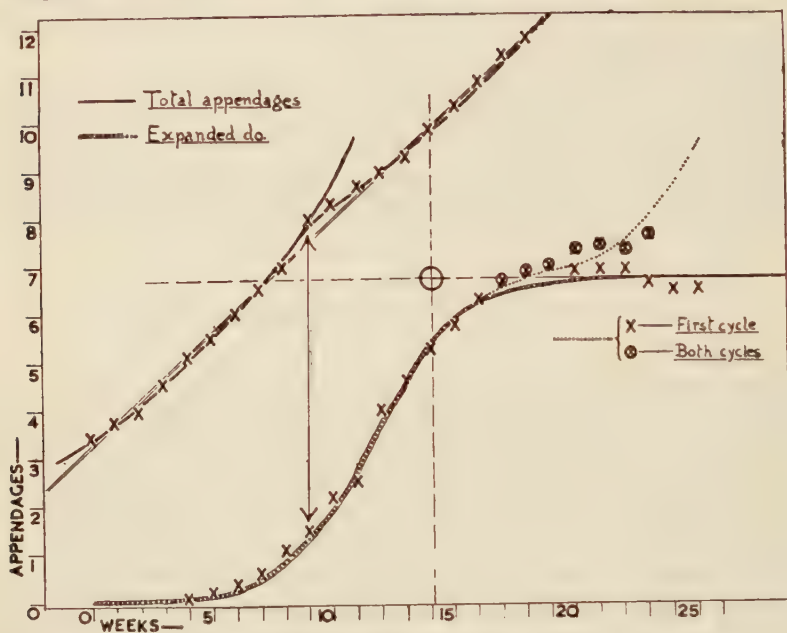


FIG. 1. 1941-2 shoots. Average appendage values plotted against weekly intervals from 0 at stage A. *Upper graph.* Total appendages, representing apical activity. *Lower graph.* Expanded appendages representing 'flushing'. The vertical arrow marks the point of inflection of the upper graph at about 9 weeks, corresponding to about 1.3 expanded appendages. The central point marked by the circle is the intersection of the observed co-ordinates for the end point of the first cycle at an average of 6.7 expanded appendages in 14.1 weeks.

being on the expansion of the last leaf of the cycle; but owing to variations in the duration of the *banji* and flush periods, and consequent overlapping of the two cycles, the 'flushing' points show a relationship with time which, if continued for a number of cycles, would consist of a smooth sequence of sigmoid curves. Actually, the data for first-cycle shoots only can be fitted closely to the logistic curve (see below) represented by the equation

$$y = \frac{6.7}{1 + 741.3e^{-0.5710x}}$$

The *total* appendage values, representing bud activity, fall very nearly on a straight line, the calculated regression for y on x from weeks 0 to 18 inclusive being $y = 3.30 + 0.47x$. This linear function indicates a constant average apical activity rate of about 0.5 appendages per week or a plastochron of 2.12

weeks per appendage. The theoretical appendage value for stage A ($x = 0$) is 3.3 appendages. The regression passes very close to the observed end-point for the first cycle at $y = 10.2$, $x = 14.1$. The chief departure from the straight line regression occurs at about stage E, between 8 and 9 weeks from the origin of the x -axis. However, this is very slight and suggests an inflexion

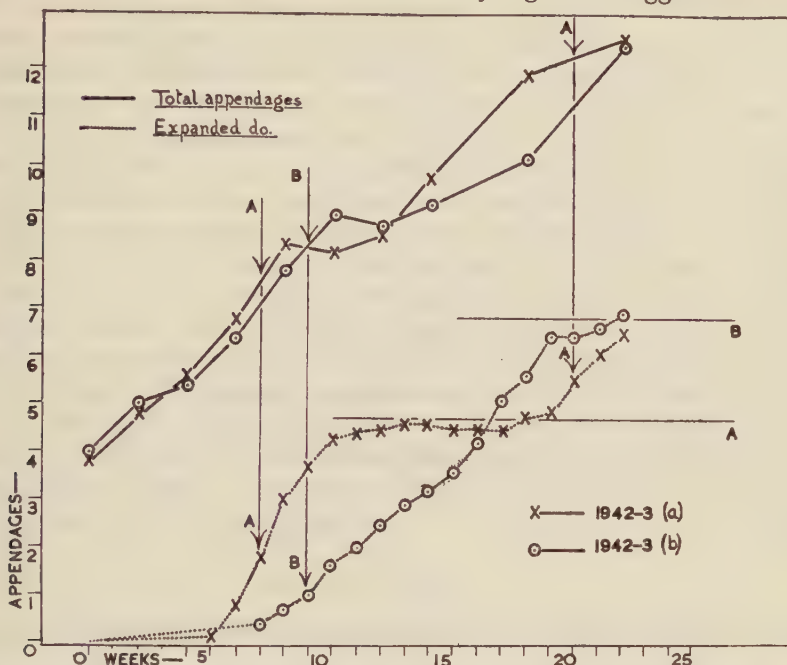


FIG. 2. 1942-3 shoots. Apical activity and flushing, (a) and (b) bushes. *Upper graphs.* Total appendages from averages of 5 buds by dissection at 2- and later 4-week intervals. The vertical arrows mark the points of inflexion as they would occur if the graphs were 'smoothed': they are extended to the corresponding values for expanded appendages. *Lower graphs.* Expanded appendages from averages of all available shoots, weekly. The horizontal lines mark the average first cycle totals of 4.7 and 6.8 expanded appendages, respectively.

only rather than any actual discontinuity as would result from the occurrence of a 'resting' stage. This is further brought out by graduating the points from 0 to 9 weeks inclusive to the exponential expression $y = 3.36e^{0.097x}$ which here gives at least as close a fit to the observed data as the straight line regression. Even so, and with due allowance for the 'smoothing effect', the graph as a whole is strongly suggestive of the continuity and relative constancy of apical activity from one flush cycle to another.

1942-3 shoots. The (a) bush gave an average flush cycle of 4.7 expanded appendages in 10.2 weeks, the (b) bushes 6.8 appendages in 16.6 weeks. It was in view of this difference that the two lots of shoots were kept separate, reducing the size of sample to 5 buds only at each occasion out of 10 dissected. The results are shown in Fig. 2, where the upper graphs give the average appendage values from the dissections at 2- and 4-week intervals, the lower

graphs the average flushing stage computed weekly from all the shoots including those sampled. Both sets of data agree well with the 1941-2 observations. The flushing curves are again sigmoid, although the longer cycle in the (b) bushes tends to obscure this. No 'smoothing effect' would be expected in the total appendage value graphs owing to the smallness of the individual samples. Both sets of points lie very roughly along a straight line indicating an average plastochron of some 2.4 weeks and both again show a point of inflexion, nearly at stage F in the (a) bush (possibly also at E' in the second cycle) and at stage E in the (b) bushes. These graphs appear to emphasize the inflexion into an actual discontinuity so that a resting stage could be postulated between 8 and 10 weeks in (a) and between 10 and 12 weeks in (b). With due allowance for sampling errors, a general impression of the continuity of apical activity remains with the possibility of an interruption not exceeding one plastochron at some time between stages E and F.

Combined data. Since all the observations are based in the first place on the recognition of separate, i.e. discrete, stages in development, the truest picture of flushing behaviour and of apical activity alike is probably obtained for the 'average shoot' by averaging times against stages of development rather than stages against arbitrary time intervals, as heretofore. This was done by combining all the data for the 73 shoots which completed at least the A to A' cycle in the form of weighted averages as shown in Table II. From these averages, Fig. 3 was constructed: it is based on an average shoot of 5.9

TABLE II

Summary of Appendage Values and Time Intervals for Successive Flush Stages

Stage.	1941-2		1942-3 (a)		1942-3 (b)		Weighted averages.	
	Appendage value.	Weeks from A.	Appendage value.	Weeks from A.	Appendage value.	Weeks from A.	Appendage value.	Weeks from A.
A . .	3.5	—	4.1	—	4.1	—	3.8	0
B . .	4.0	2.2	4.7	2.6	4.4	2.4	4.3	2.4
C . .	4.7	3.4	5.4	4.4	5.4	3.7	5.1	3.8
D . .	6.5	6.1	6.3	6.2	6.4	6.1	6.4	6.1
E . .	7.3	8.5	7.3	7.1	8.3	8.8	7.4	(1) 8.0
F . .	9.0	10.1	8.0	8.0	8.8	10.7	8.6	(2) 9.3
F+ . .	9.0	11.2	8.4	8.7	9.5	12.2	8.8	(3) 10.3
G . .	9.9	12.1	8.8	9.5	9.3	13.1	9.4	(4) 11.2
A' . .	10.2	14.1	8.8	10.2	10.8	16.6	9.7	12.9
B' . .	10.8	16.7	9.4	13.0	11.3	19.4	10.3	15.6
C' . .	11.5	18.1	10.1	15.9	12.6	20.3	11.1	17.5
D' . .	13.6	19.7	10.8	18.9	13.4	22.5	12.5	19.7
E' . .	14.4	23.0	11.8	20.4	15.0	23.6	13.4	(7) 22.0
Total for first cycle	6.7	14.1	4.7	10.2	6.8	16.6	5.9	12.9
Maximum No. of Shoots.	35 shoots		29 shoots		9 shoots		(73)	

appendages with a cycle of 12.9 weeks and is believed fairly to represent the true state of affairs in a normal, flushing shoot.

The figure may be read in both directions, the vertical or y -axis representing numbers of appendages, the horizontal or x -axis weeks from 0 at stage A.

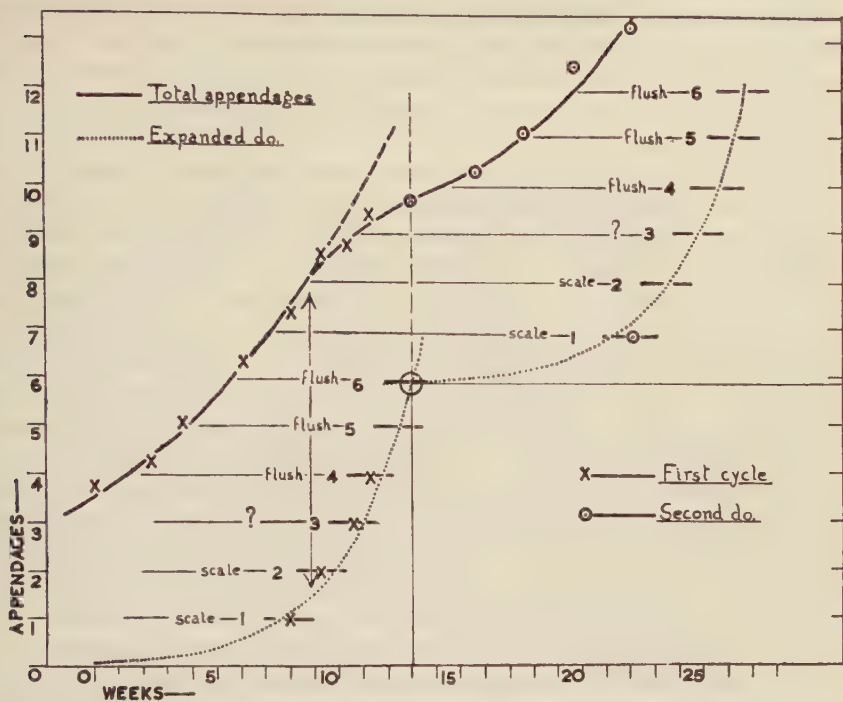


FIG. 3. Composite diagram for apical activity (upper graph) and flushing (lower graph) in the 'average' flush shoot having a cycle of 6 expanded appendages produced in 13 weeks. Data from Table II. (For further explanation see text.)

Crosses and circles indicate values for the first and second cycles respectively, from Table II.

The points representing *expanded* primordia fall very nearly on a straight line for, the first cycle, with an average interval between them (the word '*phyllochron*' may be used here) of about 1 week. However, within the given cycle the flushing process may be said to be continuous and to commence at the beginning with the gradual swelling of the bud from stage A onwards. The only true discontinuity is represented by the abrupt exposure of the new *banji* bud which marks, by convention, the beginning of a new cycle. Thus flushing is here represented as a discontinuous series of exponential curves, the observed data for the first cycle being fitted to the expression $\log y = 2.96 + 0.14x$ or $y = 0.091e^{0.322x}$ and a similar curve being erected for the second cycle by eye. These curves appear to give an adequate representation of the gradual swelling of the bud and unfolding of the first scale leaves and the more rapid subsequent expansion of the foliage leaves previously noted

(cf. Bond, 1942, Table I). Further justification for this view will be found in the subsequent treatment of the growth in length of the flush shoot. Keeping to whole numbers, the *first* and *second*, *seventh* and *eighth* appendages are labelled as scale leaves, the *fourth*, *fifth*, *sixth*, and *tenth* and subsequently as foliage leaves. The *third* and *ninth* appendages are not labelled: they might conveniently be considered as fish leaves, although clearly defined fish leaves occurred only in the 1941-2 shoots. It seems likely that their absence, in the 1942-3 (*a*) shoots, at least, may be characteristic of the growth produced early in the pruning cycle.

The points representing *total* primordia again emphasize the continuous nature of apical activity. They fall very approximately on a straight line corresponding to a regression, or average plastochron, of about 2.2 weeks per appendage. The *average* rate of apical activity throughout the period of observation is, therefore, about 0.45 primordia per week. When graduated to a smooth curve, however, they give a truer picture of apical activity as a fluctuating process. Thus, the data from stages A to F inclusive, in the first cycle, give a close agreement with the expression $\log y = 0.552 + 0.04x$ or $y = 3.56e^{0.092x}$. The continuous curve, in order to pass through the end-point of the first cycle at $y = 9.7$, $x = 12.9$ (cf. Table I), departs from the calculated exponential function, the point of inflexion being at 8.8 weeks as shown by the arrow in the figure. Thereafter, during the second cycle, the exponential form of the curve is again resumed. Referring to the curve for flushing, below, it is apparent that the inflexion of the apical activity curve corresponds to the value of 1.5 expanded appendages. In other words it occurs about the beginning of the active elongation of the flush shoot.

It was stated that the figure could be read in both directions. Thus, the *vertical* distance between the upper and lower curve (at least from stage E onwards) shows the average number of initials actually enclosed in the bud at any time, the *horizontal* distance between them the time taken by the several appendages from their origin at the apex to their ultimate expansion. Figure 3 therefore epitomizes the entire vegetative development of the flush shoot.

Mutual relationships.

The production of new primordia by the apical meristem can now fairly be viewed as a continuous process, varying slightly in rate according to the stage in the flush cycle. In each cycle the rate of apical activity tends to increase logarithmically from stage A to a maximum at the beginning of active flushing at about stage F. Thereafter it declines until the shoot again 'goes *banji*', when the phase of logarithmic increase again commences. It becomes of interest to define the relationship between apical activity and the flushing process more precisely. This can be done by means of the correlation diagram, Fig. 4.

The diagram shows the relationship between apical activity rate (*y*-axis) and flushing rate (*x*-axis) jointly for the three sets of data from 73 shoots, as summarized in Table I. For each set of data, graphs were prepared as in Fig. 3. From the three graphs values for each available primordium were then

obtained, for entry into Fig. 4. Apical activity rates were computed graphically by measuring the 'slope' (appendages produced per week) of the *total* appendages curve. Flushing rates were measured directly from the corresponding values for *expanded* appendages—the 'flushing' curves being exponential, the rate of flushing is proportional to the stage reached $dy/dx \sim y$.

In each series of observations two phases can be distinguished. In the

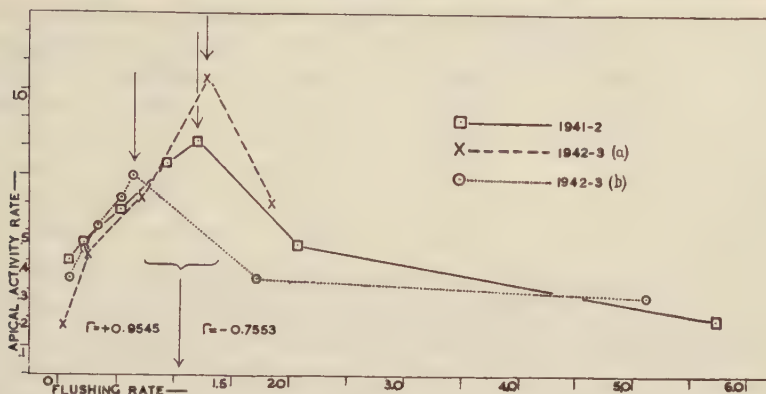


FIG. 4. Correlation diagram for apical activity rate and flushing rate. Both rates are expressed as appendages per week. The arrows show the 'limiting values' (above, actual; below, the average), separating the phases of positive and negative correlation corresponding to the values of r on the two sides of the arrow below.

first phase, activity rate and flushing rate show a strong positive correlation ($r = +0.9545$, $n = 12$). This relationship is maintained up to a limiting value of x , as shown by the arrows in the diagram. In the second phase, from the limiting value onwards, activity rate and flushing rate show a negative correlation ($r = 0.7553$, $n = 6$). The two correlation coefficients are significant at $P < 0.01$ and < 0.05 respectively. The first phase corresponds to what is usually described as the *banji* period, from stage A to E (the average of the limiting value for x , referred to above, being 1.04 expanded appendages, or 1.17 by weighting, as in Table II). During this time what is here referred to as 'flushing' is represented chiefly by the swelling of the bud—there is little or no active expansion. Thus, the two processes which are positively correlated are likely to be of the same, predominantly meristematic, nature and a positive relationship between them is in accordance with expectation. It is the later, negative correlation between apical activity and 'flushing' in the usually accepted sense of active elongation and expansion that is significant, since there is now a fundamental divergence in the nature of the two processes, 'flushing' in this sense involving little or no meristematic activity. This point will be referred to again later, but it is worth noting here that the *banji* bud of tea is by no means in the 'resting' condition. In the intervals between flushing, the bud is undergoing its most active development, and if there is any period during which the bud may be said to 'rest' at all, it is at the time of greatest apparent outward activity.

192 Bond—*Studies in the Vegetative Growth and Anatomy of the The production of scale and flush primordia.*

At the time of greatest apical activity, at stage E or later, the total number of appendages is always in excess of that required for the current flush. This is shown below in Table III and will be made clear by reference to Fig. 3.

TABLE III

Numbers of Appendages at the Times of Greatest Apical Activity

	Total appendages.	Still in bud.	Already expanded.	Average for current flush.	Excess over current flush.
1941-2	9.5	8.0	1.5	6.7	2.8
1942-3 (a)	7.4	6.2	1.2	4.7	2.7
" (b)	8.0	6.9	1.1	6.8	1.2
Combined (from Fig. 3)	8.1	6.6	1.5	5.9	2.3

It is this excess of appendages that represents the scale primordia of the following cycle. In other words, the scale primordia are laid down faster than the flush (foliage leaf) primordia. Measured in terms of the plastochron or interval elapsing from the origin of the primordium *preceding* the one in question, the average values from all data are as follows, viz. scale primordia, 1.6 weeks: flush primordia, 2.6 weeks. It should be remembered that 'scale' and 'flush' primordia are here designated as such only from their relative positions, omitting doubtful cases. Thus, in the 1941-2 shoots, with an average cycle of 6.7 appendages (see Table III), the seventh appendage will be more often a flush leaf than a scale; the eighth appendage, on the average, can fairly safely be taken as a scale, and so on. This difficulty is inherent in the adoption of a sampling technique to replace direct observation and is encountered throughout; however, it is not held to affect the general validity of the conclusions reached. In the present case, for instance, the difference between the plastochrons for scale and flush primordia is likely to have been underestimated rather than otherwise.

The question of the 'determination' of the scale and flush primordia, i.e. of the age at which they are first distinguishable with certainty, receives further consideration in the next section of this paper.

GROWTH AND 'DETERMINATION' OF THE PRIMORDIA

Applicability of the logistic curve.

As a means of calculating growth rates from measurements obtained by sampling at different stages in the flush cycle, extensive use has been made of the Verhulst-Pearl logistic curve (Pearl, 1940). This is an expression in the form of $y = K / (1 + Ce^{-rt})$ where y is the quantity measured and K is the maximum or upper asymptote of y , as used here, the average length at maturity of the organ which is being measured. All calculations have been set out as in the edition of Pearl's book cited. The growth constant r is determined from the regression $\log_e K - y/y = \log_e C - rt$. In practice, it is convenient to

work with common logarithms, plotting the successive values of $\log K-y/y$ against time and fitting the best straight line by eye (l.c., p. 462). The slope of this line is then the constant m , where $r = 2.30258m$. The logistic curve is a sigmoid one and in the early stages (or as K approaches infinity) is indistinguishable from the ordinary exponential curve for growth by compound interest. As Pearl (l.c., p. 461) says, 'the constant r is the inherent rate of growth . . . , and . . . this rate diminishes with time. That the rate does not hold to the inherent value of r is a result of the damping effect of the factor $(K-y)$, which measures the aggregate of forces that slow down and finally stop the growth.'

Certain preliminary illustrations of the applicability of the logistic curve to the growth of tea may now be given.

Growth of the primordia by plastochrons. In the apical bud the *youngest* primordium can be considered, on the average, as about in the middle of the *first* plastochron of its existence, the *next* youngest in its *second* plastochron, and so on. Thus, if the growth rates of the primordia can be assumed to be approximately constant, the average lengths of successive primordia from the youngest (i.e. innermost) outwards will be equivalent to the lengths reached by a given primordium in successive intervals of time as measured in plastochrons. In this manner, from a single sample, the whole or at least a major part of the growth of the 'average primordium' can be computed.

Ideal conditions for this are provided by aperiodic 'leader' shoots (see Bond, 1942, p. 609) in which a long succession of flush leaves is produced without any *banji* intervals and all stages of growth are available from the first plastochron to maturity. Such conditions were realized in a batch of ten shoots examined in November 1942, from the bush from which the 1942-3 (a) shoots were obtained shortly afterwards. These shoots were very uniform, the apical bud consisting of six leaf-initials and the fifth or sixth expanded leaf being apparently fully mature and lignified. The average time from origin in the first plastochron to maturity was 10.8 plastochrons. The close agreement between the observed growth of the leaves and their internodes by plastochrons and the calculated, logistic curves is shown by Figs. 5-6. As would be expected, agreement is least close in the last few plastochrons shortly before maturity, since at this stage the influence of climatic and other factors external to the leaf or internode is probably greatest. The values of K were necessarily determined from mature leaves and internodes farther down on the same shoot and this, too, probably introduces a slight inaccuracy at the end of the curve.

The uniformity of this material leads to the suggestion that the plastochron here remains more or less constant and equal to the 'phyllochron' (see above). In other words, it seems likely that as soon as a leaf is unfolded from the bud, a new initial replaces it at the apex, so that the number of initials in the bud remains constant.

Logistic curves were similarly computed from a second batch of leader shoots, and for the cataphylls and foliage leaves separately in the 1941 bud

dissections. A summary of the logistic growth-rates (as m) obtained from these sets of data is given in Table IV, together with the logarithmic growth

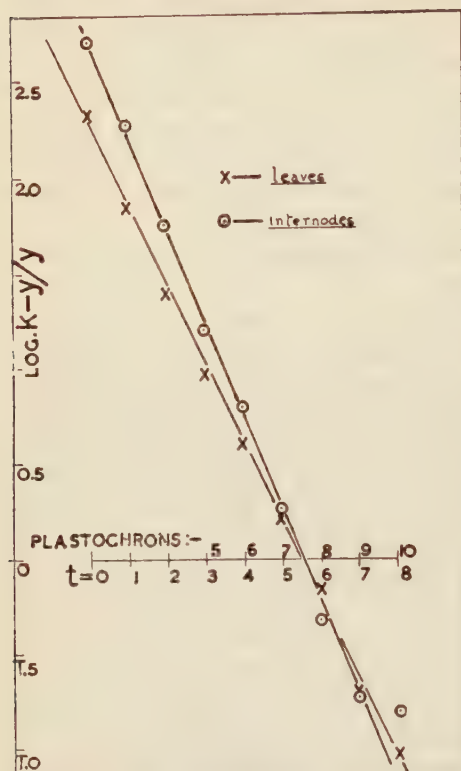


FIG. 5. $\text{Log}(K-y)/y$ plotting for the growth of flush leaves and their internodes, by plastochrons (see Fig. 6).

rates obtained by plotting the logarithms of appendage or internode lengths against time, up to the fifth plastochron inclusive. The agreement between these and the logistic rates exemplifies the meaning of the quotation from Pearl (1940) above.

The average plastochron for the 1941 material is about 2.1 weeks (see above). The average of the logistic growth-rates for both types of appendage in these buds is 0.4418 per plastochron, or 0.2104 per week which corresponds to an arithmetic value of 1.623. The rate of increase in length is, therefore, about 62 per cent. per week.

Growth of the whole shoot. An attempt was also made to express the growth of the shoot as a whole by means of the logistic curve, using the 1942-3 data. The average lengths of the stem were calculated for the successive flush stages from the beginning at stage A to maturity at stage C'. These were then plotted against the average time intervals from stage A as given in Table II.

The results are shown in Fig. 7 which, with the inset giving the $\text{log}(K-y)/y$ plotting, shows that agreement with the calculated expressions is fairly close

TABLE IV

Logistic and Logarithmic Growth-rates per Plastochron

Data.	Logistic growth-rate 'm'			Logarithmic growth-rate		
	Leaves.	Inter-nodes.	Average.	Leaves.	Inter-nodes.	Average.
Flush leaves—1942	0.4143	0.4940	0.4540	0.4508	0.4608	0.4558
aperiodic shoots, 1st lot						
Ditto, 2nd lot	0.4914	0.5371	0.5142	0.5131	0.5082	0.5106
Scale leaves—1941						
dissections . . .	0.4220	0.4975	0.4598	—	—	—
Flush leaves—1941						
dissections . . .	0.4378	0.4100	0.4239	0.4234	—	—

for the (b) bushes but not so good for the (a) bush which has a shorter flush cycle and a more rapid rate of growth. Again, the value for K cannot be computed from the shoots actually dissected, and there is considerable departure from the calculated curve in the last few weeks. Probably growth would better be represented by a skew curve of the type shown in the figure, the

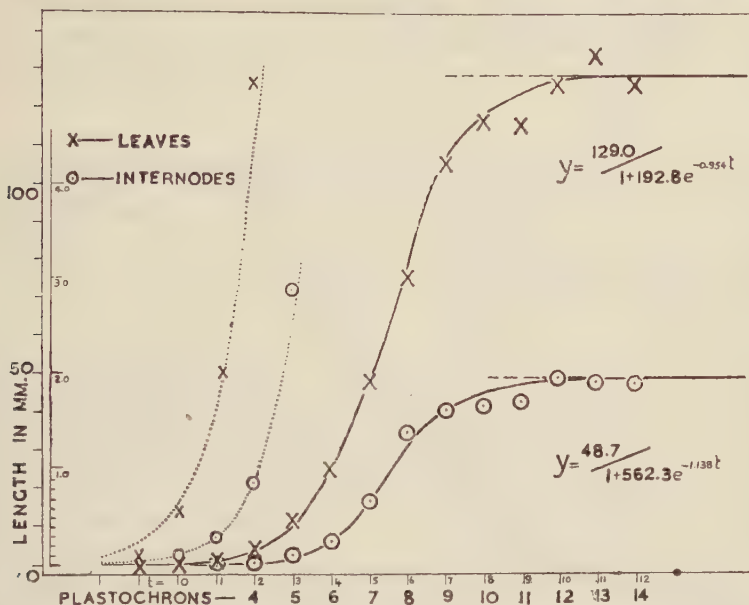


FIG. 6. Logistic curves for the growth of flush leaves and their internodes, by plastochrons. The dotted lines show the early part of the two curves on twenty-five times the vertical scale (inset). The graphs are based on the $\log(K-y)/y$ plottings shown in Fig. 5.

more abrupt inflexion of which would reflect the more or less abrupt termination of the current flushing cycle to which reference has already been made. The values of the growth constant m for the two lots of shoots corresponds to weekly growth rates of 93 per cent. and 38 per cent. for the (a) and (b) bushes respectively. It may be of significance that the average of these two values ($m = 0.2125$, giving about 65 per cent. per week) is close to the average rate of growth of the primordia as previously determined from the 1941 dissections.

Appendages and internodes.

Inherent in the concept of the apical bud as a succession of 'unit primordia' (Bond, 1942, p. 616) is the view that the young leaf-initial is hardly separable from the internode below it. On anatomical grounds, especially in the occurrence of the single, broad leaf trace, a close mutual relationship between the appendages and their associated internodes would be expected throughout the period of their development.

Correlations in length. In the mature flush shoot, a general agreement is maintained between the lengths of the scale, fish, and flush leaves and their

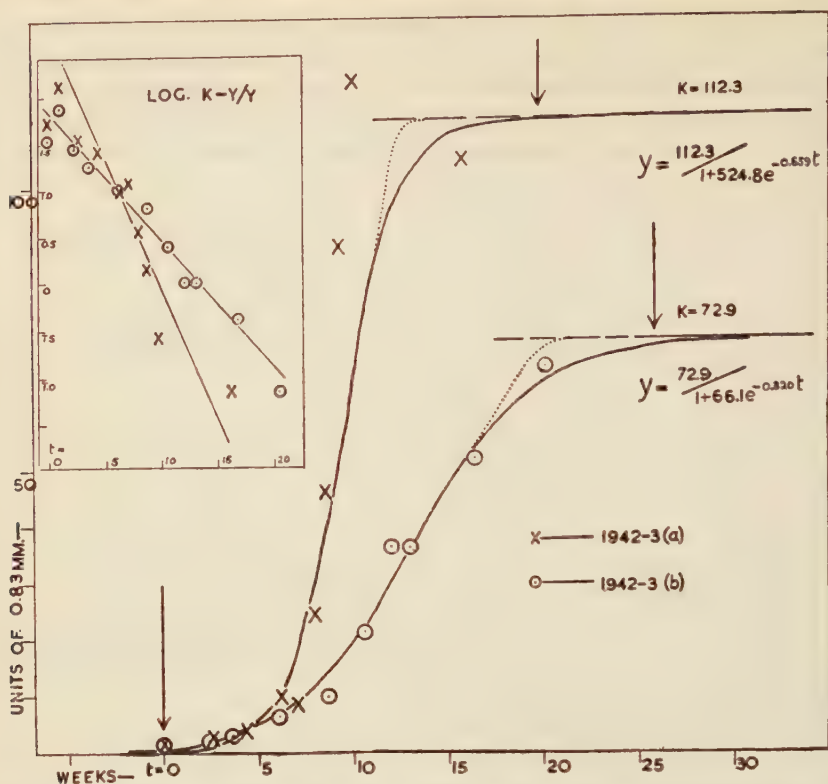


FIG. 7. Growth in length of the flush shoot. The continuous lines are the calculated logistic curves corresponding to the equations given. The dotted lines represent the more probable 'skew' curves making allowance for the more or less abrupt cessation of growth at the end of the flush period. The vertical arrows mark the probable beginning and end of the effective period of growth, namely, 20 weeks for the (a) bushes, 26 weeks for the (b) bushes. Inset. The $\log(K-y)/y$ plottings from which the curves were constructed.

TABLE V

Lengths of Mature Appendages and their Internodes (in mm.)

	Average No. in shoot.	Average length (mm.)		Ratio of lengths.
		Appendages.	Internodes.	
Scale leaves	4.0	12.6	2.2	5.7 : 1
Fish leaves	1.3	32.4 (20-60)	9.3	3.5 : 1
Flush leaves	3.8	108.9 (40-162)	20.1	5.4 : 1

Whole shoot: 9.1 appendages, length 97.3 mm.

internodes, respectively, as shown by Table V which is based on 317 shoots recorded between November 1941 and April 1942. The combined ratio of appendage length to internode length, from Table V, is 5.3 : 1.

Within the bud, or during the first few plastochrons only in the life of the primordia, high correlations are obtained between appendage and internode lengths (e.g. $r = +0.9570$, $n = 53$), the value of the correlation coefficient

decreasing slightly as the later developmental stages are included. In some shoots, especially where the bud is included together with mature foliage leaves and scales or where two or more flush periods are combined, quite low values are obtained (e.g. $r = +0.3657$, $n = 44$). As would be expected, high values are obtained for a leaf and its internode in a given position, e.g. as the last leaf of the cycle, although even here there can be considerable variation. This can be illustrated by the data for the last flush leaf (appendage 'o') of the 1942-3 shoots, as shown in Table VI.

TABLE VI

*Particulars of the last Flush Leaf and its Internode, 1942-3 Shoots
(Mature Stages only)*

Bushes.	Range of size (mm.)		Average leaf: internode ratio.	Correlation coefficient r between leaf and internode size.
	Leaves.	Internodes.		
(a)	51-159	5-43	5.3 : 1	+0.8570 ($n = 49$)
(b)	70-155	9-37	5.1 : 1	+0.8480 ($n = 54$)

Correlations in growth rate. A general correspondence in the growth rates *per plastochron* of flush and scale leaves and their internodes is apparent from Table IV. Table VII, below, gives *weekly* logistic growth rates, m , computed from the 1942-3 observations. From these data, the following correlation coefficients were determined, viz.:

(a) shoots: $r = +0.8783$
 (b) shoots: $r = +0.8263$ } joint data: $r = 0.8345$ (all significant at $P < 0.01$)

The regression coefficients, for growth rates of appendages on internodes, were in no case significantly different from unity.

From Table VII it is apparent that the growth rates of the leaves are nearly always in excess of the corresponding values for internodes. The average weekly growth rates for leaves and internodes, from all data, are 0.233 (71 per cent.) and 0.177 (50 per cent.) respectively, the difference between them being significant by 't'-test at $P < 0.01$. The meaning of this result is not clear, although a possible hormonal interpretation may be postulated.

The growth rates of successive primordia.

For the 1942-3 shoots, logistic growth-rates were computed for the leaves and internodes individually for each primordium in turn, according to its position in the flush cycle as already described. The results are given in detail in Table VIII and a selection is presented graphically in Figs. 8-9.

The results show that in each lot of shoots, *the primordial growth-rate increases from the first primordium onwards, reaching a maximum with the last flush leaf of the cycle and again falling abruptly to a minimum with the first primordium of the new cycle.* That this happens in both lots and with cycles of different lengths is sufficient evidence that the fluctuation is an inherent one and is not merely a reflection of some environmental change. The conclusion emerges that *the primordia are distinguishable by their growth rates*, those with

TABLE VII
Logistic Growth-rates per week ('m') of Successive Primordia

Primordium No.	1942-3(a)					1942-3(b)				
	Designation.		Logistic growth rate.		Period of observation (weeks).	Designation.		Logistic growth rate.		Period of observation (weeks).
	Previous flush		Leaf.	Internode.		Previous flush		Leaf.	Internode.	
0			0.411	0.264	0-2 (K = 102.4)			0.332	0.185	0-4 (K = 106.5)
1	1st scale		0.176	0.145	0-8 (K = 18.0)	1st scale		0.143	0.128	0-6 (K = 18.0)
2	2nd scale		0.278	0.164	0-9 (K = 25.4)	2nd scale		0.155	0.123	0-8 (K = 22.4)
3	1st flush		0.331	0.188	0-10 (K = 102.4)	? *		0.150	0.145	0-8 (K = 47.6)
4	2nd flush		0.334	0.245	2-10 0.6-68.1	Flush		0.188	0.187	1-10 0.5-27.1 (K = 106.5)
5	3rd flush		0.391	0.374	2-10 0.7-45.5	Flush		0.224	0.180	2-10 0.1-16.7
6	1st scale		0.141	0.050	6-22 1.2-15.9 (K = 25.4)	Flush		0.266	0.224	4-10 0.2-6.5
7	—		—	—	—	? **		—	—	—
8	—		—	—	—	Scale		0.104	0.110	8-18 0.2-3.0 (K = 18.0)
						Scale		0.111	0.124	10-18 0.3-1.4 (K = 22.4)

The size ranges are those actually recorded during the period of observation of each leaf respectively. Weeks are counted from the first sample at '0' on 9. xii. 1942.

* This leaf was a scale 0.7 times and a flush leaf 0.3 times, on the average. A composite value of K was calculated accordingly.

** Either the last flush leaf or the first scale leaf of the next cycle. See Fig. 8.

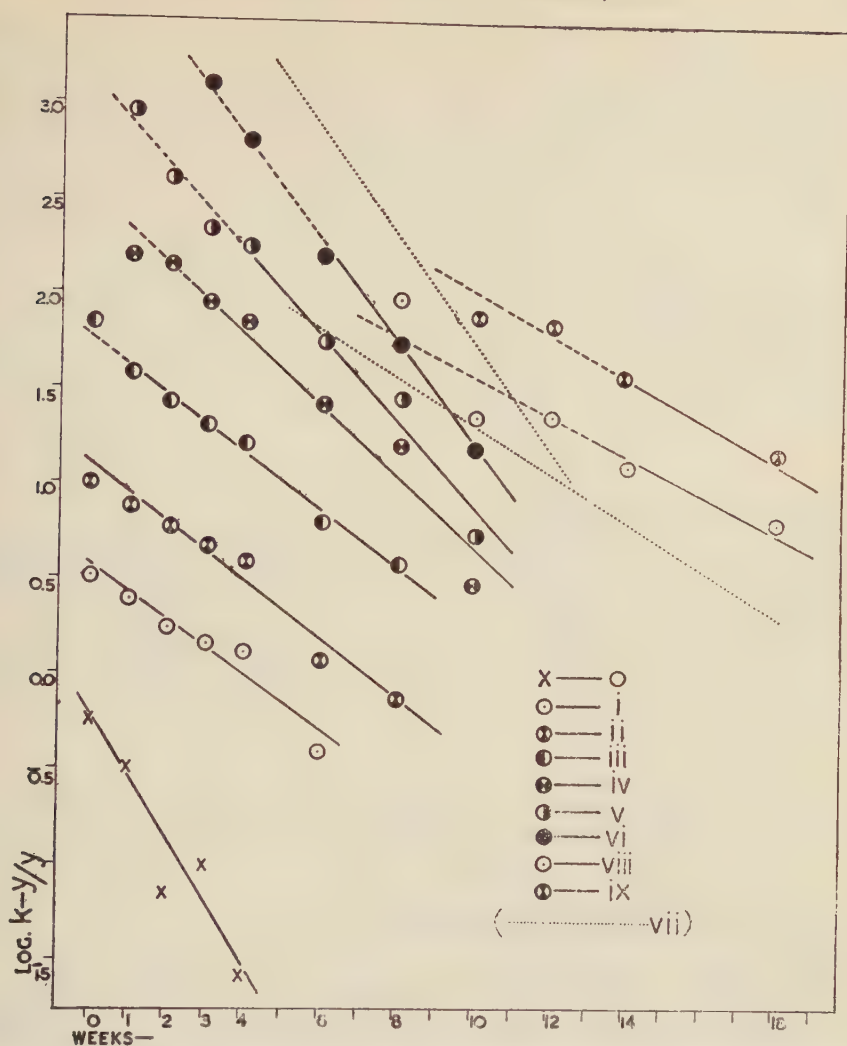


FIG. 8. $\text{Log}(K-y)/y$ plottings for successive appendages, 1942-3 (*b*) shoots, beginning with the previous flush leaf 'o' and ending with leaf 'g', the second or third scale of the second cycle (cf. Table VII). The points represent observed data, from which the lines are drawn in by eye, the 'slope' of the lines, successively, giving the logistic growth-rates m , as in the table. The *interrupted* portions of the lines refer to the possible 'indeterminate' phase of growth of the appendages, prior to their third plastochron. Appendage '7', in these buds, was sometimes a flush-leaf initial (i.e. the last of the cycle), sometimes the succeeding outer scale-leaf initial. The two *dotted* lines have been drawn in to fit the limited data available for these two categories: they give values of m as 0.282 (flush) and 0.124 (scale). Week 'o', in this and the subsequent figure, is counted from the first sample dissected on 9. xii. 1942.

a relatively low growth-rate developing into scale leaves and those that grow more rapidly into foliage leaves. Here also, although in fact not shown clearly by these shoots, is a ready 'explanation' of the nature of the fish leaf, as an appendage intermediate in growth rate between a cataphyll and a foliage

leaf. Fig. 9 shows the calculated curves and observed data for appendages 0, 1, and 6 in the 1942-3 (b) shoots plotted against actual intervals of time from December 9, 1942. The derivation of the curves from relatively limited observations towards the end, the middle, and the beginning of the primordial

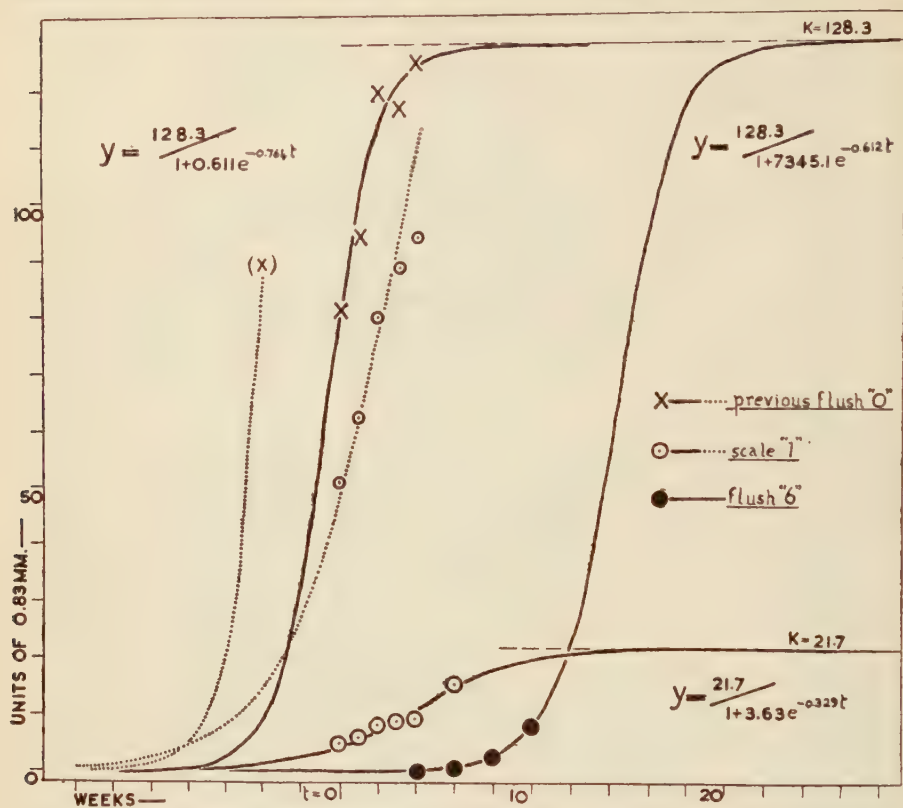


FIG. 9. Logistic curves for the growth of various appendages, 1942-3 (b) shoots, corresponding to the $\log K - y/y$ plottings in Fig. 8. The dotted lines show the early part of the growth of the last flush leaf 'o' and outer scale leaf '1' to 10 times the vertical scale of the rest of the figure.

growth-cycle respectively is well seen. For purposes of illustration the two extreme types of growth only have been included in Fig. 9, although, as Fig. 8 and Table VII show, the change in growth rate is progressive and continuous. However, although as a rule no two primordia exhibit precisely the same growth rate, average rates can be calculated for scales and foliage leaves, respectively, as follows, viz. scale leaves $m = 0.139$; flush leaves $m = 0.270$. The difference is significant by t -test at $P < 0.01$. These, on converting to arithmetic growth-rates, give for scales an average weekly increase of about 38 per cent., for flush leaves 86 per cent. The average from all data gives $m = 0.205$, or a weekly rate of increase of 60 per cent. which is very close to the estimated value for the 1941 material, from the 'plastochron' calculations (see above).

Comparing the successive change in primordial growth-rates with the fluctuating rate of apical activity, already established, it will be apparent that the same gradual rise to a maximum and abrupt fall to the minimum again, in each complete flush cycle, is shown by both values. However, the two fluctuations are not coincident, as is shown by the fact that it is those primordia which are laid down at the time of *greatest* apical activity which proceed to develop at the *lowest* individual growth-rates to produce cataphylls rather than foliage leaves. A simple inverse relationship would also appear to be precluded by the asymmetric nature of the fluctuation in both cases. The question arises at this point as to whether the primordia are 'determined' by their respective growth-rates from the start, or whether, in fact, the distinction between them becomes apparent only at some later stage of their embryonic development.

The early phases of primordial growth.

As revealed by bud dissections at the comparatively low magnifications employed, and in the absence of histological observations to the contrary, the young primordia at all positions in the flushing cycle are indistinguishable in appearance up to the end of their second or the beginning of their third plastochron. That is, the two or three innermost initials of all buds examined have been, on the average, of the same relative size.

Table VIII makes this point clear for the 1942-3 (*b*) shoots. The table

TABLE VIII

Lengths of the Three Innermost Leaf-initials of the Bud in Successive Stages. 1942-3 (b) shoots. (0.083 mm. units)

[illegible]

is entered with the average lengths (in the original micrometer units) of the three innermost initials as determined from the successive samples of 5 buds each. The true position of the initials is indicated, allowance being made for the varying total appendage values of the buds, by placing each bud (or shoot) on a common base line corresponding to the outermost scale primordium. Thus the innermost primordium of an n -leaf bud becomes the second from the apex in an $(n+1)$ -leaf bud, and so on.

The figures can be combined to show the growth of individual initials at weekly intervals from their first plastochron. The averages of these initial lengths (y), plotted logarithmically against time in weeks (x), give a close agreement with the regression $\log y = 0.347 + 0.210x$ (see Fig. 11). In other words, growth in length is exponential and can be represented by the equation $y = 2.22e^{0.484x}$. Similarly, the equation for the 1942-3 (*a*) shoots becomes $y = 2.65e^{0.461x}$. From these, arithmetic values can be derived which give an average increase of about 60 per cent. per week (cf. Table IV, &c.). By this method of calculation $x = 0$ corresponds to the *middle* of the first plastochron. Average values of y for the *end* of the first plastochron (x now = *c.* 1.2 weeks) and the *end* of the second plastochron ($x = c. 3.6 weeks) are about 0.4 mm. and 1.1 mm. respectively.$

The early growth of the primordia can also be estimated by means of the calculated logistic curves. As an example, the curves for the last flush leaf 0 and the succeeding scale leaf 1 of the 1942-3 (*b*) data can be used, as in Fig. 9. In that figure both curves are plotted against real units of time measured from a fixed date. The actual interval separating the origin of these two primordia, as determined from the plotting of total appendage values against time for the 1942-3 (*b*) shoots (cf. above, and Fig. 3, &c.), is about 1.6 weeks. Therefore, the early part of the two curves can be redrawn with this difference in time eliminated, to show the growth of the primordia on the same *relative* time scale. This has been done in Fig. 10. The two curves intersect at a value of y corresponding to a length of about 0.94 mm. and thereafter quickly diverge. Going back in time, both curves are asymptotic to a zero value of y , without again intersecting. Some modification of them is, therefore, necessary if they are to give a true picture of the actual manner of growth of the initials. By previous definition, the smallest recognizable leaf-initial has been assumed to have a length of a single micrometer unit of 0.083 mm. To bring the two logistic curves to a common starting-point representing this value they have been combined by plotting average values of y for successive intervals along the x -axis backwards from the point of intersection. The resultant curve is represented by the dotted line in the figure, and the origin is now fixed at that position which corresponds to a value of $y = 0.083$ mm. on this curve. With reference to this origin, the intersection of the two original curves at $y = 0.94$ mm. now corresponds to a value of $x = 5.4$ weeks, as computed graphically. Furthermore, the 'average' curve, from $y = 0.083$ at $x = 0$ to $y = 0.94$ at $x = 5.4$, when plotted logarithmically, reveals an essential similarity to the expression previously derived from a logarithmic

treatment of the early stages of growth as calculated from the average of all available primordia (Fig. 11).

The same result was also obtained from the data for the 1942-3 (a) shoots as shown in Table IX.

Thus the calculated (logistic) and observed (logarithmic) data are in

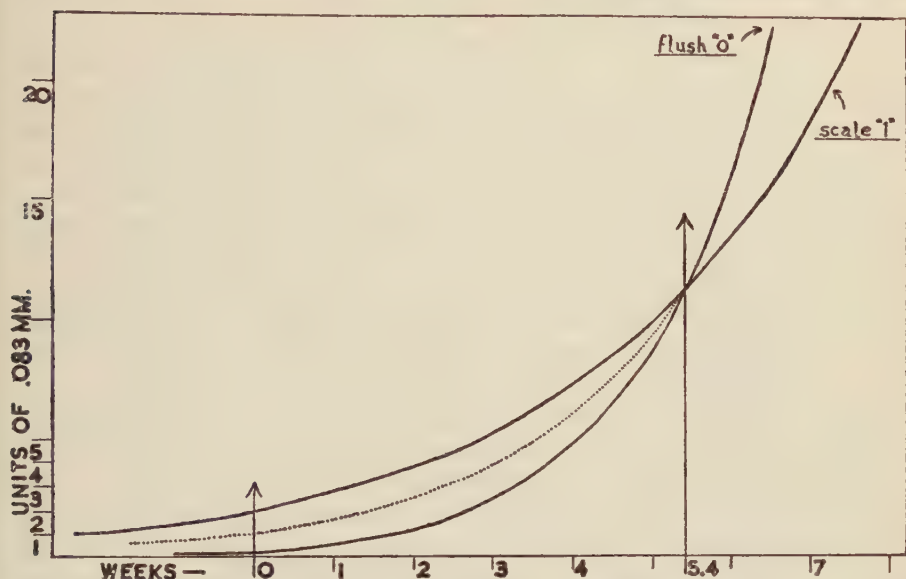


FIG. 10. Early part of the logistic curves for the last flush leaf 'o' and the outer scale leaf '1', reduced to the same *relative* position in the time scale (from Fig. 9). The dotted line represents the average of the two solid curves. The vertical arrows mark the origin (by definition) at $y = 1$ unit of 0.083 mm. and the point of intersection at $y = 0.94$ mm., 5.4 weeks later.

TABLE IX

Data for Early Growth of Leaf-initials

Material.	Logarithmic growth-rate, from 'average' of logistic curves for previous flush and 1st scale leaves.	Logarithmic growth-rate as previously determined from observed data.	Intersection of logistic curves.	
			y (mm.).	x (weeks).
1942-3 (a)	0.222 per week	0.200 per week	1.21	5.0
1942-3 (b)	0.179 "	0.210 "	0.94	5.4
Average values	0.200 "	0.205 "	1.08	5.2

sufficiently close agreement to point to a common exponential expression as an adequate representation of the early growth of the several leaf-initials (hence, presumably, of the primordia as a whole) up to the stage indicated by the point of intersection of the calculated logistic curves, as in Fig. 10. After this stage, as the figure shows, growth is distinctly divergent, and the several logistic curves are believed to give an adequate representation of the rate and

manner of growth subsequently. In other words, the calculations as a whole lend support to the general impression that the primordia can be considered as initially 'indeterminate' and suggest a limiting size of about 1 mm. at which the developing initials reach the 'determinate' stage. The average age of 5.2 weeks from the assumed origin at which this limiting size is reached corre-

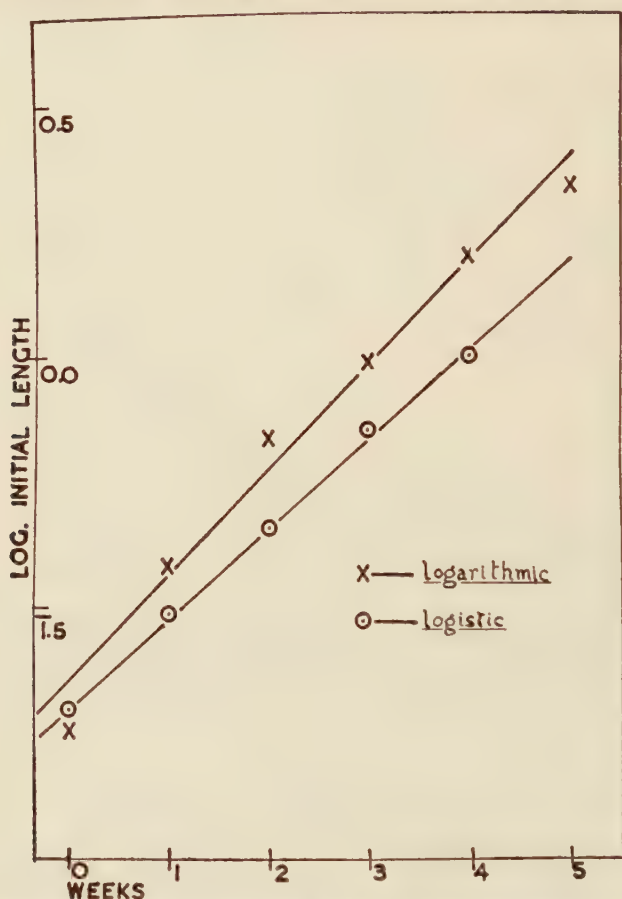


FIG. 11. Logarithmic plotting of the early growth of the leaf-initials, 1942-3 (b) shoots. X— from actual measurements, as in Table VIII, O— from the 'average' or combined logistic curve, as in Fig. 10.

sponds to some 2.2 plastochrons on the average as previously determined, which, again, places the 'determinate' stage at about the beginning of the third plastochron.

That in some instances the logistic growth-rates characteristic of the later stages of primordial growth are equally applicable from the earliest origin onwards cannot be denied. It is indicated, for example, by the size ranges in Table VII and in Fig. 8. That this is so does not alter the fact that on the evidence so far submitted to consider the primordia as 'indeterminate'

below about 1 mm. in length is at least as reasonable, if not more so, than the alternative assumption of 'determinism' from the start. Probably a study of linear growth-rates alone could never finally settle the question. Meanwhile, however, further circumstantial evidence can be obtained from the present data by a reconsideration of the relation of primordial growth-rates and apical activity.

Primordial growth-rates and apical activity.

Average primordial growth-rates for the 1942-3 shoots were determined from the mean logistic growth-rates of the appendages and their internodes successively, from Table VII. These were then plotted against apical activity rates (primordia per week) *two plastochrons later*, i.e. the growth rate of primordium 'n' against the apical activity rate at the formation of primordium 'n+2'. The two variants now show a positive correlation of $r = 0.6685$, significant at $P < 0.02$. Only 11 degrees of freedom were available for this calculation and, as Fig. 13 shows, there is reason to believe that the correlation is actually much higher than the value obtained suggests.

The position has been reached, then, that the rate of *production* of a given primordium is related to the *growth rate* of the primordium two internodes below it. While the rate of production of new primordia (i.e. apical activity rate) is constantly changing, the growth rate of the individual primordium, once established, is maintained throughout its development, the characteristic course and result of which it apparently 'determines'. Thus the growth rate, and hence the subsequent development, of successive primordia is related to the activity rate of the apex at the end of their second plastochron, again suggesting that this is in fact the 'determinate' stage in their existence.

VASCULARIZATION BELOW THE APICAL BUD

An attempt was made to estimate the probable supply of water and nutrients to the apical bud at different stages of the flushing cycle in terms of the degree of vascularization of the tissues. This was determined from outline camera-lucida drawings of hand sections of fresh material, mounted in chlor-zinc-iodine solution after clearing slightly in chloral hydrate. As far as possible, at least 5 shoots were examined of each age from stage A to stage F inclusive. Sections were cut at the base of the shoot at all ages, also at the base of the new flush growth (i.e. above the last leaf of the current flush) for the second cycle shoots from stage A' onwards. To estimate the vascular supply to the actual bud, whether flushing or otherwise, sections were also cut at an arbitrary distance of 1 cm. below the growing point (the position of which was judged by eye from the appearance of the bud). In all, 138 sections were cut and drawn from a total of 73 shoots. The drawings were made at magnifications of 35, 64, and 100 respectively, according to the size of the sections, as far as possible arranging that the whole section could be drawn comfortably without shifting the slide. In the youngest sections only the approximate

outline of the vascular (or provascular) cylinder as a whole could be drawn in; later it was possible to show the limits of the pericycle and of the xylem and phloem separately. From the drawings the percentages as detailed below were determined by means of planimeter measurements, checked in a few cases against cutting out and weighing.

The results of these observations are shown in Fig. 12 where the vertical axis represents percentages, the horizontal axis flush stages, arranged approxi-

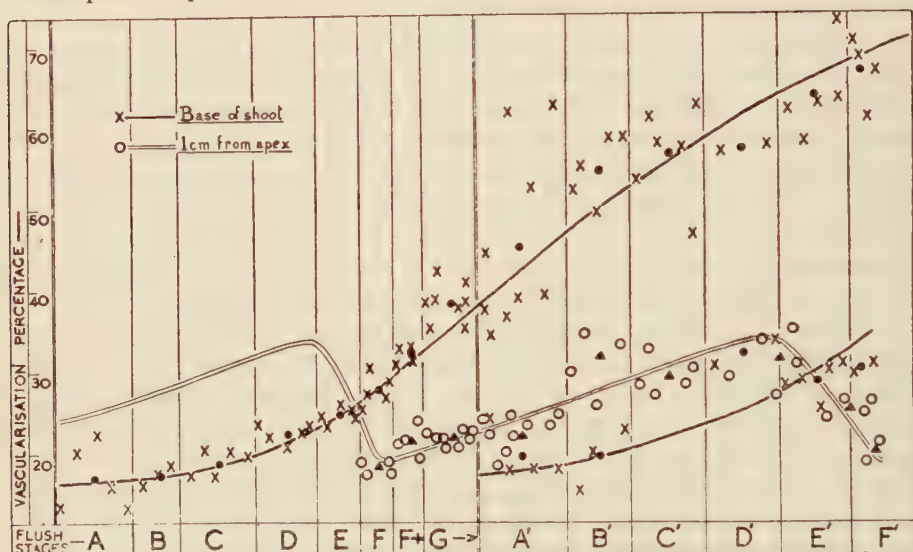


FIG. 12. Vascularization percentages as calculated from planimeter measurements of out-lines of sections plotted against flush stages. The black discs and triangles are successive average values used in drawing in the smooth curves. (For further explanation see text.)

mately to scale according to their duration in time from the average of previous data (Table II). Each point represents a single section, the arrangement of the points within the flush stages corresponding to the estimated order of maturity of the shoots from which the sections were taken. The lines have been drawn in to give a reasonable fit with the averages calculated for each stage.

The *total* vascularization percentage (including the pericycle) at the base of the shoot as a whole shows a gradual rise from about 17 per cent. at stage A to 67 per cent. at stage F'. This rise is continued subsequently until, in the old, woody shoots, nearly 100 per cent. of the cross-sectional area of the stem is vascular in the broad sense, the remains of the medulla and the thin periderm contributing a very small percentage of the whole. In the sections examined, from about stage C onwards, the pericycle remained fairly constant at an average of some 4.5 per cent. of the whole. The proportions of phloem and xylem are of interest, although they could not be distinguished with accuracy before stage D. Expressed as a percentage of xylem and phloem together, the proportion of phloem decreased from more than 60 per cent.

at stage E to about 28 per cent. at E'. Subsequent measurements, from older shoots, showed that a minimum value of about 11 per cent. is reached, the relative decrease being the result of the crushing and obliteration of the successive layers of sieve tubes.

Of greater interest than the steady increase in vascularization measured at a fixed point (i.e. at the base of a given flush period) is the fluctuating proportion of vascular tissues measured at a *given distance from the apex*. Choosing an estimated distance of 1 cm., the figure shows that vascularization (here excluding the pericycle) is at its lowest, at about 20 per cent., in stage F. At this time the rapid elongation of the flush shoot is just beginning and the bud and consequently the position of sectioning, 1 cm. below it, is being raised relative to the base of the shoot. Thus, the vascularization percentage beneath the bud, while increasing slowly, tends to diminish relative to the degree of vascularization at the base. Meanwhile the shoot has again 'gone *banji*', so that a position of 1 cm. from the apex is now *below* the last flush leaf and hence shows a *higher* vascularization percentage than the next internode above, which is immediately at the base of the new *banji* bud. This state of affairs continues until the new bud breaks into flush at stage D' to E' when, with the beginning of the new phase of elongation, the bud is once more raised up and the vascular supply to the growing point again drops rapidly to a minimum. Thus, by considering the extent of vascularization within a given distance of the apex, a periodic fluctuation has been demonstrated which is of the same nature as those previously described for apical activity and primordial growth rates.

SUMMARY OF MUTUAL RELATIONSHIPS

The relationships previously established may now be summarized in diagrammatic form, as in Fig. 13. The figure is based on an 'average shoot' of six appendages, as previously defined by Table II and Fig. 3, and consists of graphs for flushing rate, vascularization, apical activity, and for primordial growth-rate. These are set out on a common horizontal scale in unit intervals of primordia or plastochrons, with the average flush stages superimposed. Thus, from the table, stage A is presumed to extend from 3.8 to 4.3 primordia, stage B from 4.3 to 5.1, and so on. The separate graphs have been determined as follows:

(1) *Flushing rate*. The first plastochron of the new shoot is held to commence with the formation of the fourth primordium. Thus from Fig. 3 successive values for expanded appendages were read off against total appendages from the fourth onwards. These are a measure of flushing rate ($dy/dx \sim y$) in the two cycles included in the diagram.

(2) *Vascularization*. Percentage vascular tissue in transverse section, 1 cm. below the growing point, as in Fig. 12.

(3) *Apical activity*. As primordial production per week, being the differential of the 'total' appendages curve in Fig. 3.

(4) *Primordial growth-rates.* Average logistic growth-rates per week (the constant m , in common logarithms) for primordia in their third plastochron, i.e. plotted against primordia in process of formation as in graph (3). The values entered are appendage-internode means averaged for the first six

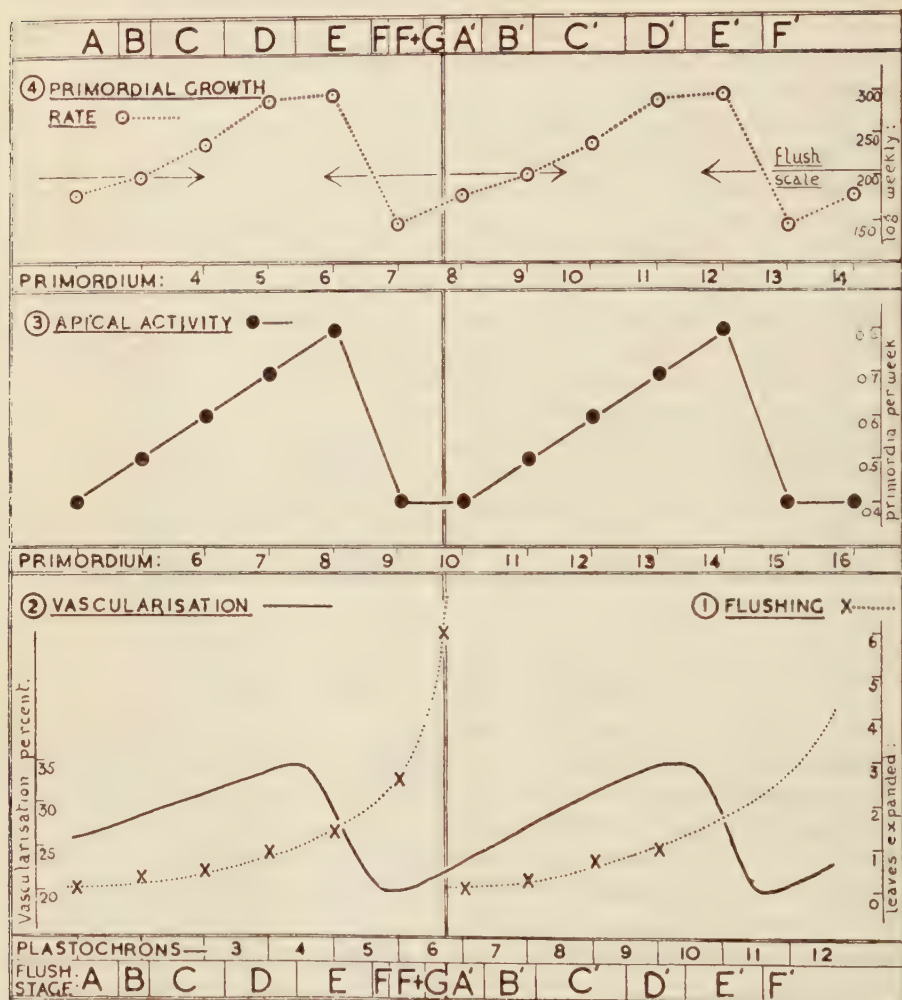


FIG. 13. A diagrammatic representation of growth relationships in the flush shoot. (For a full explanation see text.)

primordia of the 1942-3 shoots. The general average growth-rate for scale and flush leaf primordia— $m = 0.205$ per week (see above)—is here indicated as a 'limiting value' separating the two kinds of primordia. The coincidence with the determined growth-rate of the third primordium (also entered, for the next cycle, as the ninth) is interesting, since these were regarded potentially as fish-leaf initials in the 'average shoot' of Table II and Fig. 3.

As an example of the use of the graph as a whole, the middle of the 'banji period' at stage D may be compared with the middle of active flushing, stage F+. These two stages correspond to the third and the fifth plastochrons respectively of the current cycle.

Third plastochron. The bud is swollen, the first scale leaf nearly but not quite expanded ('flushing rate' *c.* 0.8). The vascular supply to the apex is approaching its maximum value of about 33 per cent. Apical activity is also nearly at its maximum with the formation of the seventh primordium, being the future first scale initial of the new cycle. Two internodes from the apex, the fifth primordium is growing nearly at the maximum rate and will subsequently develop into the second of the three foliage leaves.

Fifth plastochron. With active flushing and an average of about 2.5 expanded appendages, the vascular supply to the apex has dropped to its minimum. Concurrently, the rate of apical activity is at its lowest with the formation of the ninth primordium. Two internodes from the apex, the seventh primordium has entered its third plastochron and will develop at the lowest observed growth-rate of 0.148, or 41 per cent. per week, to form the first scale of the new cycle.

This close correspondence between the three fluctuations represented by graphs (2), (3), and (4) is sufficiently striking to suggest that, in fact, the three processes are mutually dependent. Thus, again assuming that the primordia are 'determinate' only at the end of their second plastochron, the whole sequence of events inherent in the periodic development of the flush shoot can be viewed as the expression of a consistent, self-regulating system. On this assumption, the sequence—*flushing rate, vascular supply to the bud, apical activity, primordial determination*—becomes a closed cycle. The onset of active flushing leads to a lengthening of the plastochron; this in turn reduces the growth-rate of the primordium which at the time happens to be at the right 'determinate' stage to that value which will influence its development to form a scale leaf; this slow development of scale leaves and decreased rate of apical activity breaks the sequence of expanding flush leaves so that the bud again 'goes banji'; the *banji* bud receives an increasing vascular supply and produces primordia at an increasing rate; this in turn is reflected in the increasing rate of development of the primordia to form at first perhaps a fish leaf, later, flush leaves; the rapid expansion of these and their corresponding internodes again constitutes the active process of flushing and the cycle is thus brought to completion.

A further point of interest emerges from a consideration of the fluctuating vascular supply to the bud in terms of the stages of development recognized in the previous paper of this series (Bond, 1942, pp. 615–18). The increase in vascularization from stage F to the end of stage D' now corresponds to the developmental series from stage IV–V to the 'mature' stage VII, inclusive (qq.v.). Anatomically speaking, this is characterized chiefly by the effective rise of the vascular cambium and increasing development of the metaphloem and metaxylem tissues. Thus, the fluctuation demonstrated can equally be

regarded as a measure of cambial activity, whence it appears that, within the limits of the flush shoot, a positive relationship exists between the two kinds of meristematic activity, at the apex and in the vascular cambium respectively.

DISCUSSION

So many of the relationships outlined above have been established through the use of the logistic curve to depict the course of primordial growth that some further discussion of the merits of this procedure may well precede a consideration of the results of the investigation as a whole.

The logistic curve, as Pearl (1940) shows, goes back over a century to the Belgian mathematician, P. F. Verhulst. Used from the start to describe the growth of human, and later of various animal populations, it has received comparatively little attention from botanists. It appears unfamiliar on the whole to British workers, although a paper from Pearl's laboratory (Gould et al., 1934), applying the logistic formula to the problems of seedling growth, has appeared in this journal. Recently the whole subject of growth curves has been reviewed in the new edition of Thompson's (1942) book; their botanical applications have also been dealt with by Barton-Wright (1930). From these and other sources it appears that a considerable body of literature has accumulated on the analogy between plant growth and the course of an autocatalytic monomolecular reaction, for which Robertson's equation of $\log x/(a-x) = K(t-t')$, and its differential $dx/dt = kx(a-x)$, are applicable. In these equations x and a are the equivalent respectively of y and its upper asymptote K in the logistic formula, t being the time at which $x = a/2$. Unfortunately it has not been sufficiently widely realized that the autocatalytic and the logistic curves are identical, as can easily be shown by ordinary algebraic methods. The point is made quite clear by Lotka (1925, pp. 71-6). No special significance need be attached to this—to quote Thompson (l.c., p. 258)—‘when the same curve depicts the growth of an individual, and of a population, and the velocity of a chemical reaction, it is enough to show that the analogy between these is a mathematical and not a physio-chemical one’. Pratt (1940), in the initial paper of a series dealing with the growth of *Chlorella vulgaris* in culture, makes good use of the autocatalytic equation and gives a useful list of references to previous work.

The logistic formula has been preferred in the present communication because of its greater general applicability and high predictability. Also, following Pearl's procedure, the computation, though laborious, presents little difficulty. No attempt has been made to fit ‘skew’ curves and in any case these would be of value chiefly in allowing for discrepancies in the later part of the curve, when the effect of varying external environment is more pronounced. Copeman (1928) suggested a modification of the autocatalytic equation by the introduction of a second constant to allow for the effect of external factors; more recently, the importance of climatic and other external influences on the shape of sigmoid growth curves has been emphasized for the growth of maize

by Blair (1942). On the other hand, Kramer (1943) presents data for the growth of certain conifers in North Carolina and New England, which, he says, 'serve to emphasize that the length of growing season and course of shoot growth of trees is relatively independent of normal fluctuations in environmental factors during the growing season'. Logistic curves have been fitted by the writer to Kramer's data as determined from his figures by graphical means, and in a number of cases an extremely close agreement with the calculated curves has resulted. This is interesting in view of the general similarity between the, presumed, single annual 'flush' of growth in conifers and the flush period of the tea shoot. Logistic growth-rates have also been determined with fair accuracy from Oexemann's (1942) sigmoid curves for the growth of Biloxi soybeans.

In the present communication, as in the comparison between growth-rates of internodes and appendages, and as between scale and flush primordia, lower logistic growth-rates have generally been associated with lower values of the upper asymptote K . That this is not necessarily the case is shown, for example, by the work of Albaum et al. (1940), who used the 'autocatalytic' formula in an investigation of the effect of various pre-treatments on coleoptile growth in oats and found that initially lower growth-rates were associated with higher ultimate lengths, and vice versa.

Passing to the more general aspects of this investigation, the results now obtained may be compared with the position reached in the discussion of the first paper of this series (Bond, 1942, pp. 624-6) under the general heading of apical growth and foliar determination. Compared with the previous occasion, a much clearer picture emerges of apical activity as, normally, a fluctuating process. The fluctuation occurs about the same average plastochron of 2 to $2\frac{1}{2}$ weeks. The flush shoot of tea is now seen to agree with the yearly growth of black hickory as described by Foster (1931, 1932) in producing its scale primordia at a faster rate than the primordia of the foliage leaves. The average plastochrons for the two kinds of primordia respectively are, in black hickory, 4 and $5\frac{1}{2}$ days, in tea, as now determined, about 11 and 18 days. However, the present work raises the possibility that the production of scale-leaf initials may not, in fact, be conditioned by the *rapidity* of their formation but by the relative *slowness* of the rate of apical activity a couple of plastochrons-later. Similarly, the production of foliage-leaf initials may be conditioned by the *shortening* of the plastochron subsequent to their formation, a process culminating, apparently, in the production of scale primordia later, marking the beginning of a new cycle. This possibility will stand or fall according to the result of future histological studies designed to test the assumption on which it is based, namely, that the 'determinate' stage of the primordia is reached only at the beginning of their third plastochron, when the initials themselves are nearly 1 mm. in length. In black hickory such a possibility would appear to be precluded by Foster's (1935) later studies showing that the 'determinate' stage is reached, histologically speaking, surprisingly early, when the initials are only 0.1 mm. in length and when they

are still, apparently, in their first plastochron, or at latest at the beginning of their second.

So far the discussion of foliar determination in tea has been based entirely on the behaviour of the normal, i.e. *periodic* development of the flush shoot. Meanwhile, further suggestive evidence may be afforded by considering the two types of *aperiodic* development also commonly encountered. On the one hand is the vigorous leader shoot producing a long succession of foliage leaves, in which, as noted above, it seems reasonable to assume that the *plastochron* remains more or less constant and equal to the *phyllochron*, or interval of leaf-expansion. This may be as low as a week and is in any case probably not more than the average plastochron of 11 days established for periodic flushing. On the other hand is the type of growth, associated with nitrogen deficiency (Bond, 1942, p. 609) or other conditions promoting general unthriftness, which consists merely in the indefinite production of scale leaves, without any active flushing. Here the phyllochron is considerably in excess of the value for foliage leaves and may reach a length of several weeks. The relative stability of this condition again indicates that the plastochron will be prolonged accordingly. Thus, in the two instances, where the plastochron remains more or less constant or at least shows no *cyclic* fluctuation, it is *the scale-leaf initials that are formed at the lower rate, the flush-leaf initials at the faster rate*. Rate of formation is here seen to bear a direct relationship to rate of subsequent development, and the apparent reversal of this relationship in the flush shoot follows, on the theory of foliar determination outlined above, as a necessary consequence of a fluctuating as compared with a more or less sustained rate of apical activity.

A new element is introduced into the discussion by considering the vascularization data and the relationship between apical activity and the outward stage of growth or flushing. This is the nutritional aspect, broadly speaking, and here also a comparison with the aperiodic shoots is suggestive. For if the continued production of scale leaves is a mark of nutritional deficiency, continued flushing can equally be interpreted as a result of an abnormally high level of nutrition, as would be expected to occur, for instance, during recovery from pruning and to persist in the 'leader' shoots at other times. Thus, the normal flushing cycle would seem to depend on the maintenance, more or less, of an intermediate level of nutrition. It appears, though this requires confirmation, that both the content (in appendages) and the speed (duration per appendage) of the flushing cycle would be subject to nutritional control within certain limits.

Vegetative development consists, fundamentally, of the processes of *meristematic activity* at the apex, followed by *expansion* of the tissues and organs thus laid down and, to a certain extent, by *cambial activity* in the later stages. The manner of development is an expression of the nutritional equilibrium between these three processes which, in turn, is reflected by the constancy or otherwise of the vascular supply below the apex. Thus in the aperiodic shoots of both types the equilibrium is stabilized and the vascular supply appears to

remain more or less constant accordingly, being invariably very reduced in the continually *banji* shoots, well developed in the continuously leafy shoots. In the periodic flush shoots, the equilibrium is unstable and shows a regular fluctuation as already described. Throughout the cycle there is a direct relationship between apical activity and the vascular supply to the bud; as already noted, this also points to a direct relationship between the two types of meristematic processes in the apex and in the vascular cambium respectively. On the other hand, the relationship between apical activity and outward development as measured by the flushing rate is complicated by an abrupt reversal at the end of the *banji* phase. This reversal coincides with the abrupt diminution in vascularization (and hence in cambial activity) below the apex and is expressive, besides, of the transition from a predominantly meristematic to a predominantly 'extensive' phase of development, again clearly indicating a nutritional control of the equilibrium.

The concept of the flushing cycle as a self-regulating mechanism, depending on the fluctuation in the vascular supply to the apical bud as influencing the rate of formation of successive primordia and the rate, and manner, of their subsequent development to cataphylls and foliage leaves, is thus essentially a nutritional one. The *inherent* quality of the control itself depends on the external maintenance of a level of nutrition in excess of that required for the continuous production of scale-leaves, but insufficient to permit continuous flushing. This in turn may be the result of a succession of developmental correlations which will themselves need further analysis, for example, as in Pearsall's (1923) treatment of data for the growth of the cotton plant. The whole subject of nutritional control as affecting the equilibrium between the component processes of vegetative development forms an almost unlimited field of inquiry but, in the tea plant, the present results indicate most clearly the need for further investigation of the effect of nitrogen economy and water supply. Thus, in the cotton plant, Crowther (1934) has clearly demonstrated the role of nitrogen in controlling the initiation and rate of meristematic activity and that of water in conditioning subsequent expansion. A more detailed investigation of the effect of water supply on the shape and size relationships of foliage leaves was furnished by Pearsall and Hanby (1926). As previously noted, a comparison between the periodic flushing of the tea shoot and the annual growth of woody plants, especially of conifers, may profitably be made, and in this connexion the work of Kramer (1943) and Kienholz (1934), in establishing general developmental correlations, and of Loomis (1935), in indicating the balance of meristematic and cambial activity in relation to nitrogen metabolism, is of special significance.

SUMMARY

The behaviour of the apical bud of the vegetative shoots of the tea bush under 'up-country' conditions in Ceylon has been further investigated with special reference to the rate of apical activity at different stages in the flushing

cycle and to the rate of growth of successive primordia as affecting their subsequent development into cataphylls and foliage leaves.

The apical bud has normally no 'resting period'; the production of new primordia is a continuous process which, however, shows a regular fluctuation in rate about a general average value represented by a plastochron of 2 to 2½ weeks. The period of greatest activity coincides with the beginning of active flushing, after the expansion of the first scale leaf of the current cycle. The rate of apical activity thereafter declines rapidly to a minimum and again increases slowly to the maximum in a more or less exponential manner. The outward development of the shoot from the first exposure of the young *banji* bud can also be represented exponentially, to include the gradual swelling of the bud, the more rapid expansion of the scale leaves, and the still more rapid expansion of the foliage leaves. Thus the relationship between apical activity rate and flushing rate is characterized by a phase of positive correlation during the so-called *banji* stage and beyond and a phase of negative correlation during active flushing.

Those initials which are formed by the apical bud at the period of its maximum activity later develop into the scale leaves of the succeeding flush. Thus, the average plastochron for scale-leaf initials is about 11 days, that for the foliage-leaf initials, which are produced at other times in the cycle, some 18 days.

Primordial growth-rates in tea can be satisfactorily determined from the course of growth as expressed by the Verhulst-Pearl logistic curve, $y = K/1 + Ce^{-rt}$. Examples are given of the applicability of this formula to the growth of the leaf-initials and their internodes by plastochrons and to the growth in length of the flush shoot as a whole.

There is a high positive correlation, both in dimensions and growth rates, between leaves and their corresponding internodes. Leaf growth-rates, however, are almost always higher than the internode rates, as shown by the average values (weekly growth rates) of 71 and 50 per cent. respectively given by conversion of the logistic growth constant r (or m , where $r = 2.30258m$) for all the primordia determined.

The growth rates of successive primordia in order of their formation at the apex show the same type of fluctuation as has been described above for the rate of apical activity. Thus, in an 'average' shoot of six appendages, there is a steady increase in the logistic growth-rate from the first scale-leaf initial onwards to the fifth or sixth of the cycle, with an abrupt decrease to the minimum again with the seventh appendage, representing the first initial of a new cycle. It follows that the primordia are distinguishable by their growth rates, the scale-leaf initials having a lower average growth-rate ($m = 0.139$) than the foliage-leaf initials ($m = 0.270$). The mean of these two determinations ($m = 0.205$), corresponding to a weekly increase of 60 per cent., may be viewed as a limiting value, probably characteristic of the fish leaf.

As calculated from their several logistic growth-rates, or as plotted directly from measurements obtained by bud dissection, the early growth of the

primordia during the first two plastochrons is substantially the same whatever their position in the flushing cycle. From the beginning of the third plastochron the divergence in growth is increasingly apparent. Thus, if growth is initially considered as 'indeterminate', the 'determinate' stage will be reached at about this period, which corresponds to an average initial size of some 0.9 mm. A similar conclusion is reached by considering the relation between primordial growth-rates and apical activity at various stages of the cycle, for the two fluctuations will coincide when apical activity is related to the successive growth-rates of the primordia *on entering their third plastochron*.

The supply of water and nutrient materials to the bud was estimated by measuring the percentage area of vascular (or provascular) tissue in transverse sections at a given distance, namely 1 cm., from the growing point. Here also a regular and asymmetric fluctuation was demonstrated, a maximum value, corresponding to about 33 per cent. of vascular tissue, being reached at the end of the *banji* stage, with a rapid decline to a minimum, about 20 per cent., associated with the elongation of the stem during the active flushing. Vascular supply to the bud thus varies in direct relation to apical meristematic activity as previously established. It is further shown that vascular supply as here defined can be used as a measure of cambial activity, whence it appears that within the limits of the flush shoot there is a positive relationship between the two types of meristematic process.

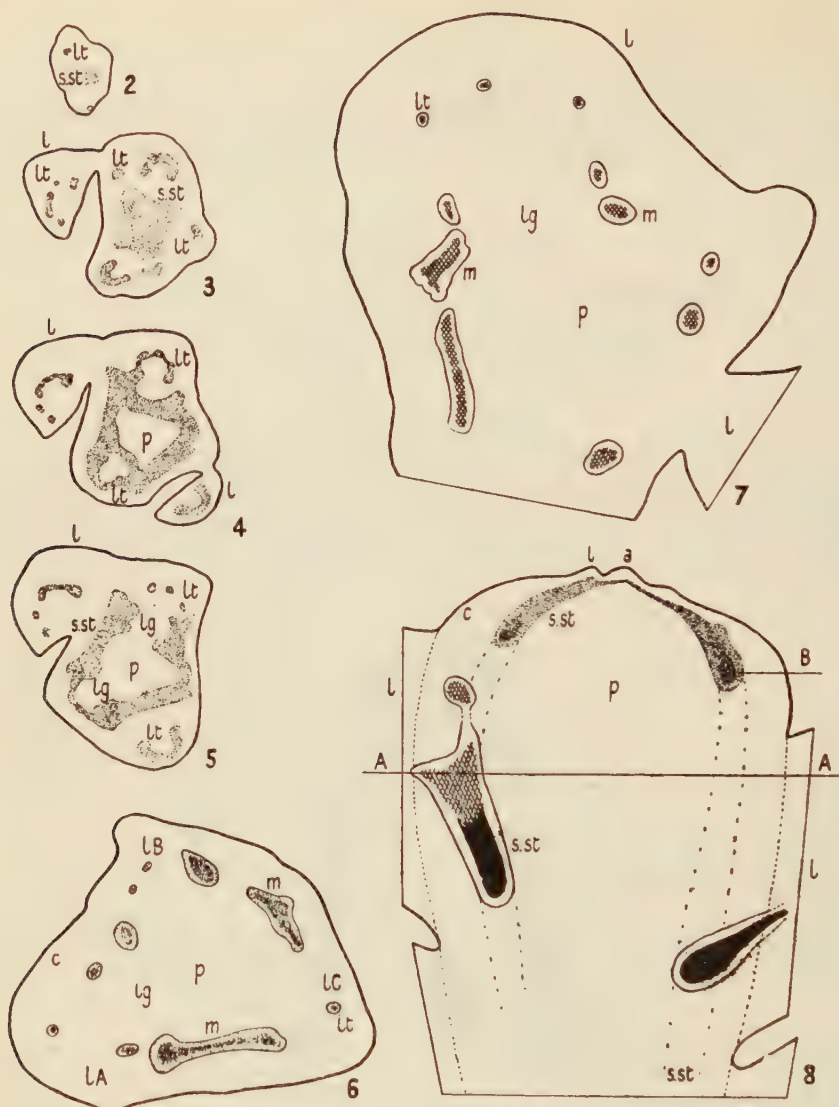
Thus, assuming that the primordia reach the 'determinate' stage only at the beginning of the third plastochron of their existence, the whole cycle of events inherent in the periodic development of the flush shoot can be viewed as a self-regulating mechanism. The onset of active flushing leads to a diminution in the vascular supply to the bud and a lengthening of the plastochron for the initials in process of formation. This in turn is followed by the 'determination' of the scale-leaf initials, the slower development of which, compared with the preceding flush leaves, breaks the sequence of expansion and the bud 'goes *banji*'. While in this condition the vascular supply is again increased, the plastochron is shortened and more young primordia receive the impetus to develop into foliage leaves so that the cycle is again renewed from the beginning.

By comparing the periodic, flushing shoots with the two types of 'aperiodic' shoot, bearing a succession of scale leaves or of foliage leaves respectively, the cycle of events described above is discussed on a nutritional basis, and further investigation of the effect of nitrogen economy and of water supply is suggested.

I am indebted to Dr. C. H. Gadd and to Dr. T. Eden for reading the manuscript and to Mr. M. H. E. Koch for the planimeter measurements.

LITERATURE CITED

- ALBAUM, H. G., KAISER, S., and EICHEL, B., 1940: Coleoptile Growth after Preliminary Soaking of Oat Grains in Various Solutions. *Amer. Journ. Bot.*, xxvii. 619.
- BARTON-WRIGHT, E. C., 1930: Recent Advances in Plant Physiology. London.
- BLAIR, R. A., 1942: Growth Rates of Maize under Field Conditions. *Pl. Physiol.*, xvii. 619.
- BOND, T. E. T., 1942: Studies in the Vegetative Growth and Anatomy of the Tea Plant (*Camellia thea* Link.) with Special Reference to the Phloem. I. The Flush Shoot. *Ann. Bot., N.S.*, vi. 607.
- COPEMAN, P. R. v. d. R., 1928: Autocatalysis and Growth. *Ann. Appl. Biol.*, xv. 613.
- CROWTHER, F., 1934: Studies in Growth Analysis of the Cotton Plant under Irrigation in the Sudan I. The Effects of Different Combinations of Nitrogen Applications and Water-Supply. *Ann. Bot.*, xlviii. 877.
- FOSTER, A. S., 1931: Investigations on the Morphology and Comparative History of Development of Foliar Organs, II. Cataphyll and Foliage-Leaf Organisation in the Black Hickory (*Carya Buckleyi* var. *arkansana*). *Amer. Journ. Bot.*, xviii. 864.
- , 1932: Investigations on the Morphology and Comparative History of Development of Foliar Organs, III. Cataphyll and Foliage-Leaf Ontogeny in the Black Hickory (*Carya Buckleyi* var. *arkansana*). *Amer. Journ. Bot.*, xix. 75.
- , 1935: A Histogenetic Study of Foliar Determination in *Carya Buckleyi* var. *arkansana* Sarg. *Amer. Journ. Bot.*, xxii. 88.
- GOULD, S. A., PEARL, R., EDWARDS, T. L., and MINER, J. R., 1934: On the Effects of Partial Removal of the Cotyledons upon the Growth and Duration of Life of Canteloup Seedlings without Exogenous Food. *Ann. Bot.*, xlviii. 575.
- KIENHOLZ, R., 1934: Leader, Needle, Cambial and Root Growth of Certain Conifers and their Interrelations. *Bot. Gaz.*, xcvi. 73.
- KRAMER, P. J., 1943: Amount and Duration of Growth of Various Species of Tree Seedlings. *Pl. Physiol.*, xviii. 239.
- LOOMIS, W. E., 1935: Translocation and Growth Balance in Woody Plants. *Ann. Bot.*, xlix. 247.
- LOTKA, A. J., 1925: Elements of Physical Biology. Baltimore.
- OEXEMANN, S. W., 1942: Relation of Seed Weight to Vegetative Growth, Differentiation, and Yield in Plants. *Amer. Journ. Bot.*, xxix. 72.
- PEARL, R., 1940: Introduction to Medical Biometry and Statistics. 3rd edn., Philadelphia and London.
- PEARSALL, W. H., 1923: Studies in Growth, IV. Correlations in Development. *Ann. Bot.*, xxxvii. 261.
- and HANBY, A. M., 1926: Growth Studies, V. Factors affecting the Development and Form of Leaves. *Ann. Bot.*, xl. 85.
- PRATT, R., 1940: Influence of the Size of the Inoculum on the Growth of *Chlorella vulgaris* in Freshly Prepared Culture Medium. *Amer. Journ. Bot.*, xxvii. 52.
- THOMPSON, D'A. W., 1942: On Growth and Form. 2nd ed. Cambridge.



FIGS. 2-8. *Dryopteris aristata*. Transverse sections through the apex of a small shoot in basipetal sequence. Vascular tissue in the initial phase of differentiation is stippled; xylem in the earliest stage of differentiation is heavily stippled; tracheides of adult size but not lignified are cross-hatched; fully differentiated xylem is shown in solid black; endodermis is indicated by a continuous line; fully developed phloem, pericycle, &c., are unshaded. Fig. 2. First indication of shoot stele (*s.st.*) immediately below the apical meristem; *lt*, leaf-trace. Fig. 3. The initial vascular tissue is disposed as an unbroken ring round a central pith; the vascular tissues of leaf primordia at different stages of development and disruption are also shown. Fig. 4 shows the enlarging pith (*p*) and the insertion of leaf-traces on the shoot stele. Fig. 5. The vascular ring is now interrupted by the insertion of the enlarging vascular systems of two leaves, i.e. leaf-gaps (*lg*) are present. Fig. 6. The dictyostelic condition is now established, wide gaps being associated with the insertion of the leaves, *la*, *lb*, *lc*; at this level the first

system notable changes take place and internal structural features of considerable complexity may be produced. Hitherto the vascular system in ferns has been investigated chiefly by the method of sectioning shoots from the base upwards. The limitations of this method and the need for a re-examination of stelar development from the apex backwards at successive stages in the ontogenetic development have already been indicated (Wardlaw, 1944). For just as the morphology of the leafy shoot results from the integrated activities of the shoot apex together with the successive leaf apices so, too, the vascular system of the plant is a composite whole which originates behind the several meristems. A re-examination of stelar development from this point of view is the object of the present investigation.

THE DEVELOPMENT OF THE VASCULAR SYSTEM

On proceeding from the shoot apex downwards in protostelic ferns the initial vascular tissue undergoes further differentiation and development and yields a compact, non-medullated cylindrical stele, to the periphery of which, with a minimum of disturbance, the developing leaf-traces become conjoined. In larger protosteles a central parenchymatous pith may be present. This condition is normally to be observed in the development of sporelings of solenostelic ferns, the adult stele being of cylindrical shape with a central pith and inner endodermis. This otherwise continuous cylinder is interrupted at intervals by leaf-gaps, usually consisting of parenchyma. The dictyostele is simply a special case of solenostely in which several leaf-gaps are present in any transverse section of the shoot.

Transverse sections of the apex of a dictyostelic fern, e.g. *Dryopteris aristata*, cut in basipetal sequence yield the following successive observations, Figs. 2-8: (i) the three-sided apical cell and adjacent segments; (ii) an undivided central area of vascular tissue, i.e. the shoot stele in the initial stage of differentiation, surrounded by cells of the apical meristem, Fig. 2; (iii) an uninterrupted ring of vascular tissue, enclosing a central pith and surrounded by the developing tissues of cortex and epidermis, Figs. 3, 4; (iv) an interrupted vascular ring, the gaps being associated with developing leaf primordia, Figs. 5-7. If, as in certain young squat petiole buds of *Dryopteris filix-mas*, no leaf primordia have been differentiated, the solenostelic condition indicated in (iii) above persists over a certain longitudinal region of the shoot.

Figs. 2-7 not only indicate that the origin and progressive widening of the

evidence of differentiation into phloem and xylem can be observed. Fig. 7. At this level phloem and xylem are differentiated but the tracheides (cross-hatched) are still un lignified. Fig. 8. Longitudinal section through the distal region of a similar young plant showing the basipetal differentiation and expansion of the vascular system. Level *A* corresponds approximately with Fig. 7 and level *B* with Fig. 6. Figs. 2-5 lie between the base of the apical cell and level *B*. It will be noted that towards the base of the region of expansion, where the adult diameter is almost attained, the differentiation of phloem and xylem is just becoming perceptible. *l*, leaf; *c*, cortex; *m*, meristele. ($\times 15$.)

leaf-gaps are directly related to leaf development, they also demonstrate important facts concerning the vascular system of leaf primordia. Thus in very young primordia, Figs. 2, 3, as also immediately below the apex in older primordia, the initial vascular tissue (as seen in cross-section) consists of a single semicircular or elliptical strand, which becomes conjoined with the shoot stele without the development of a leaf-gap (see also Fig. 1). In older leaf primordia the vascular tissue, still a single strand, becomes crescentic or horseshoe-shaped, the free margins being thrust apart by a central development of parenchymatous tissue; at its conjunction with the shoot stele a small gap of developing parenchymatous tissue is now seen to interrupt the otherwise continuous vascular ring, Fig. 5. In still older primordia the crescentic mass becomes subdivided into 3, 4, and eventually 5 separate strands or leaf-traces, with developing parenchyma between. The 'diameter' (chord) of this multiple leaf-trace system becomes conspicuously enlarged during development, and at the conjunction of the leaf-traces with the shoot stele increasingly conspicuous foliar gaps become evident. The consequential disruption of the shoot stele by the overlapping of leaf-gaps is accompanied by a progressive enlargement of the parenchymatous pith within. Finally, with further growth and differentiation the characteristic morphology of the adult dictyostelic shoot becomes evident. A transverse section thus shows a large pith, widely separated meristeles, large leaf-bases with widely separated strands, and a relatively narrow cortex, Fig. 7. But it may be emphasized that these remarkable developments all proceed from the relatively simple structural arrangements found at the shoot apex, Figs. 2, 3.

DEVELOPMENT OF THE LEAF-TRACE IN DRYOPTERIS

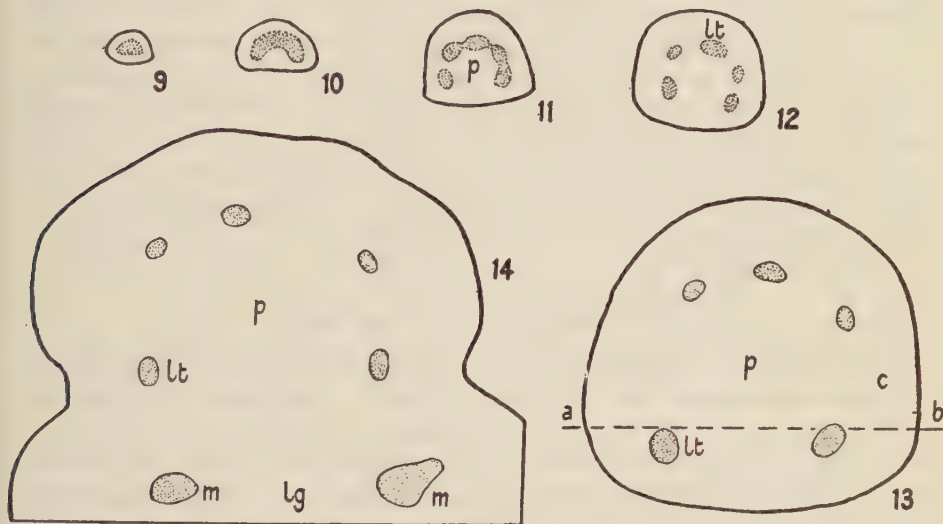
The transverse sections of leaf primordia in Figs. 9–13, each taken about the level where the adaxial surface joins the shoot, show the initial crescentic outline of the vascular tissue and its progressive fragmentation during development. A similar series could also be obtained by sectioning a somewhat developed primordium from the apex backwards. This breaking up is associated with a parenchymatous development of regions of the crescentic mass, the further growth and enlargement of this parenchyma together with the expansion of the pith being responsible for the pattern of the widely spaced leaf-traces seen in the adult leaf-base.

If, during the growth of the bilaterally symmetrical leaf primordium, the enlargement of the crescentic mass of vascular tissue does not keep pace with the developing parenchymatous pith within, it is inevitable that stresses will develop in it at various points. In species of *Dryopteris* it is a fact of observation that the rate of growth of the pith is considerably greater than that of the vascular tissue. Hence the inevitable disruption of the crescentic mass during development. On the other hand, in ferns such as *Osmunda regalis* the enlargement of the vascular tissue keeps pace with that of the pith and no disruption takes place. In *Dryopteris*, in relation to the aforementioned

stresses, local groups of cells initially differentiated as vascular elements are modified in their subsequent development; they enlarge, divide, and develop into typical parenchyma, and this growth together with the contemporaneous enlargement of the pith, particularly in the tangential plane, results in a wide separation of the individual leaf-traces. These developments exercise an important influence on the internal morphology of the shoot.

RELATION BETWEEN LEAF DEVELOPMENT AND STELAR MORPHOLOGY

The several constituent tissues at the shoot apex in *D. aristata* are in a semi-plastic condition, and the newly differentiated stele, as seen in transverse



FIGS. 9-14. *Dryopteris aristata*. Transverse sections of leaf primordia at different stages of development, each section being taken at the level where the adaxial surface was about to join the shoot. Vascular tissue, stippled; p, pith; lt, leaf-trace; lg, leaf-gap. ($\times 24$.)

section, consists of a ring of undifferentiated vascular tissue with pith and cortical parenchyma developing internally and externally respectively, Fig. 3. As Priestley (1928, p. 13) has pointed out, vascular tissue in this position is under an increased pressure because of the enlargement of the parenchymatous tissue on either side. Provided no other factors intervened to disturb the equilibrium it might be anticipated that during growth the vascular cylinder in such a plastic, radially symmetrical system would show a progressive enlargement and possibly reduction in thickness but would not become disrupted. This, in fact, has been borne out in experimentally induced solenosteles (Wardlaw, 1944a). Here it may be noted that the bilateral symmetry of the leaf primordium permits of a lateral, i.e. tangential, distension and disruption of the vascular tissue in a manner not found in the shoot. Now the insertion of the leaf-trace system on the shoot stele constitutes an equilibrium-disturbing factor: in relation to the tangential distension of the leaf-

base a stress is set up in the adjacent tissue of the shoot stele. The constitution of the cells affected is modified in such a manner that they no longer develop as vascular elements. In fact, potential vascular tissue continues its development as parenchyma and so gives rise to a leaf-gap.

The view that a direct relationship exists between leaf development and the internal morphology of the shoot has thus far been based on the observation of normal changes during development. But it has been demonstrated in species of *Dryopteris* that if a number of successive leaf primordia are destroyed at a very early stage, the vascular system of the shoot develops, not as a dictyostele, but as a continuous uninterrupted cylinder, i.e. as a solenostele (Wardlaw, 1944a). Such a solenostele is seen to be an acropetal extension of the dictyostelic cylinder present in the normal, untreated region of the shoot, with the leaf-gap parenchyma replaced by vascular tissue; or, more precisely, tissue which was initially differentiated at the apex as vascular tissue has, in the absence of modifying factors associated with leaf development, become fully developed as vascular tissue. In those instances where a leaf primordium had grown to a slightly larger size before being destroyed, a small leaf-gap developed, the width of the gap being in direct proportion to the 'diameter' of the vascular crescent in the leaf-base.

THE DEVELOPMENT OF PARENCHYMA

The several parenchymatous regions, cortex, leaf-gap and pith, constitute notable components of the internal morphology of the shoot: they become increasingly conspicuous during the ontogenetic development and progressively less conspicuous when the shoot diminishes in size as in 'starvation' experiments. It is therefore relevant to examine, even in the most general terms, the factors which may be concerned in their development and to consider how such developments affect the internal morphology of the leafy shoot.

Cortical parenchyma originates by the division by periclinal and anticlinal walls of superficial cells which originally formed part of the apical meristem. Leaf-gap parenchyma as indicated above originates, not as part of the primary differentiation at the apical meristem, but as a subsequent development. This 'parenchymatization' of vascular tissue has already been recognized by other workers (Kostytschew, 1922; Priestley, 1928); the process involves a real transformation of cells which otherwise would have become normal elements of the stele. The origin of pith parenchyma is very similar: it develops *within* the cone of vascular tissue that lies immediately below the apical meristem, and again involves a modification of newly differentiated vascular tissue. The young parenchymatous cells in both leaf-gap and pith continue to grow and divide and thereby give rise to the mass of parenchyma in those regions. At maturity parenchyma from the three regions is so alike as to be indistinguishable. But with regard to the factors operative in the production of this tissue our knowledge is singularly inadequate.

THE RELATIVE DEVELOPMENT OF PITH AND CORTEX

The pattern to be observed in any transverse section of a fern shoot is determined by the relative proportions and positions of the pith, vascular tissue, leaf-gaps, and cortex. The pattern changes not only from the base of the sporophyte upwards into the adult region but also from the apex downwards. The extent of development of parenchyma is important in the production of a particular pattern. The pith, for example, may be only slightly or very extensively developed and the appearance of the cross-section thereby correspondingly modified. Now during the apical growth of a well-developed plant such as *Dryopteris aristata*, considerable quantities of nutrient substances must be supplied from below. Figs. 6-8 show that, in the sub-terminal region, although both cortex and pith are already quite well developed, the vascular tissue which lies between them is still in the initial phase of differentiation. It is therefore cogent to inquire if this vascular tissue is the means whereby metabolites are translocated and distributed, centrifugally and centripetally to cortex and pith respectively, and to the apical meristem, or whether, alternatively, the growing region is supplied by upward diffusion over the whole cross-sectional area. To answer this question would undoubtedly be to effect a notable advance in our knowledge of formative processes at the apex. With this end in view various experiments are now being undertaken. Meanwhile data are given below which indicate how the internal morphology of different organs is affected by the relative development of pith and cortex.

Tubers of Nephrolepis cordifolia

The leafless tuber of *Nephrolepis cordifolia* begins as a protostelic stolon with no pith, but on further development and with the accumulation of storage materials the protostele opens out into a wide dictyostelic system; this closes again as the apical end of the tuber is approached. The following measurements (5 times natural size) refer to the thin stolon at the base of the tuber and to the broadest region, Fig. 15 (after Sahni) :

					Radius (mm.)	Cross-sectional area (mm. ²)
Protostelic stolon	{ Stem	.	.	.	4.2	55.0
	{ Stele	.	.	.	1.5	7.0
	{ Cortex	.	.	.	2.7	48.0
Tuber	{ Stem	.	.	.	27.0	2260.0
	{ Pith	.	.	.	18.0	1004.0
	{ Cortex	.	.	.	9.0	1256.0
Radius cortex/pith = 0.5					Cross-sectional area cortex/pith = 1.26	

The radius of the cortex is that of the stem less that of the stele.

Thus during the enlargement of a non-medullated stolon into a tuber, the cross-sectional area of the stem has increased about 40 times. In the widest region of the tuber the cross-sectional area of the pith is almost equal to that

of the cortex; in a slightly larger tuber sectioned by the writer the two areas were equal. In both morphological regions large deposits of starch are the rule. Evidence of this kind might suggest that the development of both pith

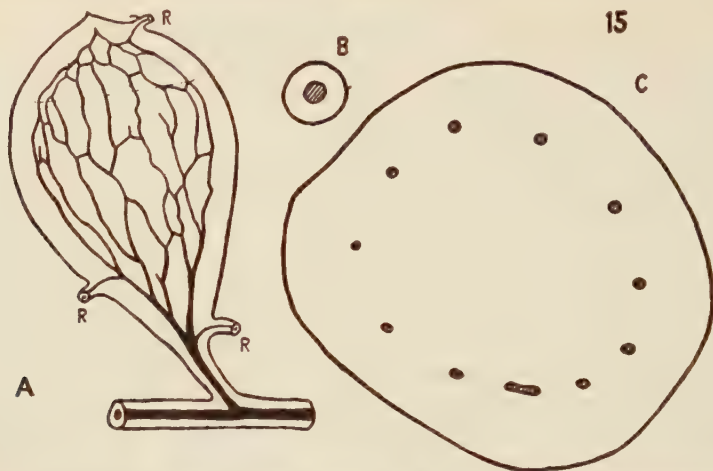


FIG. 15. *Nephrolepis cordifolia*. (After Sahni.) A, stolon bearing a tuber in which the protosteles enlarge and become dictyostelic and then contract again at the apex; B, transverse sections of protostelic stolon and tuber respectively. ($\times 5$.)

and cortex is referable to the centripetal and centrifugal diffusion respectively of metabolites from the vascular system, but another conclusion is also possible.

Shoots of Cyathea, Dicksonia, &c.

In sporeling plants of *Cyathea dealbata* the vascular system which is protostelic at the base becomes medullated and thereafter solenostelic and dictyostelic. Near the apex of such a plant the undifferentiated vascular tissue is seen in cross-section as a continuous uninterrupted ring, as in *D. aristata*, Figs. 2, 3. Measurements of the radii of stem, cortex, stele, and pith at different successive levels have been used to obtain the ratios shown in Fig. 16. Above the level of the solid protostele a conspicuous feature of the developing shoot, apart from the leaf-gaps, is the progressive enlargement of the pith. Thus the ratio of the stem radius to the stele radius falls from 3.3 at the base to 1.7 at the widest region of the shoot, and the corresponding ratio *radius cortex* (R_c) to *radius pith* (R_p) falls from 8.1 to 0.8; for the same levels the ratio *cross-sectional area cortex* (A_c) to *cross-sectional area pith* (A_p) falls from 132 to 2.8. In the obconical development of the shoot, which is seen to be largely related to the increase in diameter of the pith, the ratio $A_c : A_p$ falls to 1.6 in the dictyostelic region below the apex where the xylem is still undifferentiated. Nearer the apex these ratios increase again, the development of the pith tending to lag behind that of the cortex.

In de Bary's illustration of *Cyathea imrayana* the radius of the shoot is 27.0 mm., the ratio $R_c : R_p$ is 0.5, and the ratio $A_c : A_p$ is 1.7. A large stem

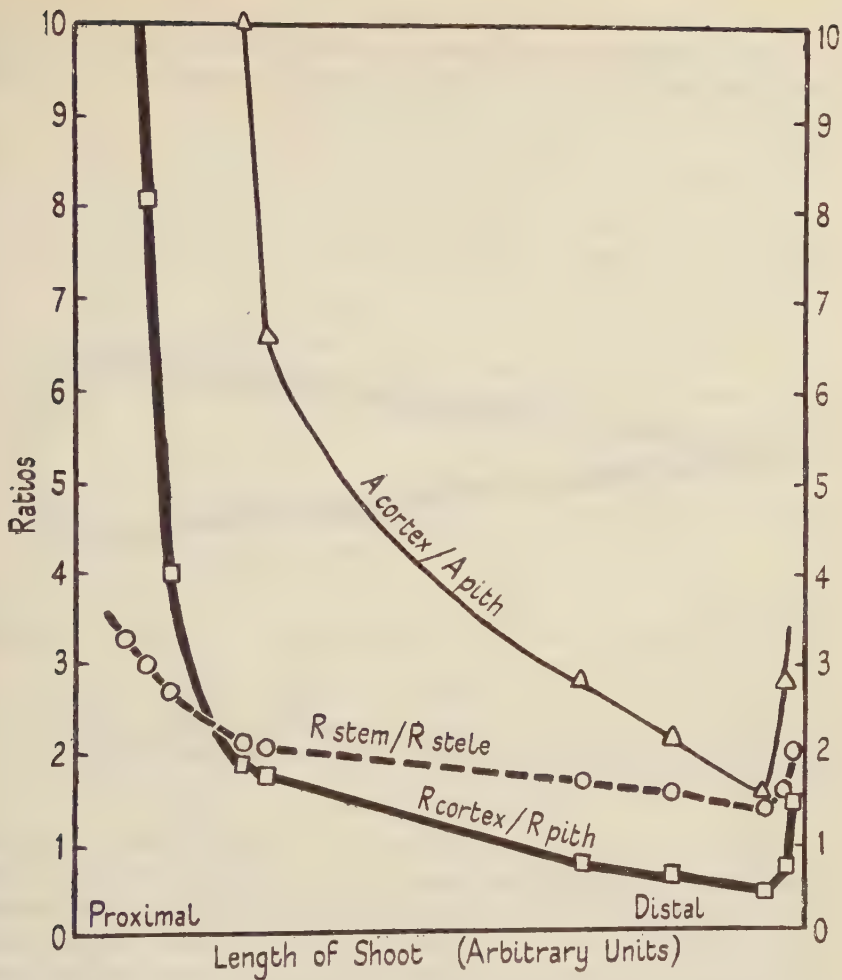


FIG. 16. *Cyathea dealbata*. Curves showing ratios relating to the pith, stele, and cortex during the development of a young sporophyte plant. R , radius; A , cross-sectional area. The ratios at the right-hand side, i.e. those referring to the apical region of the shoot, are only approximately accurate.

of *Cyathea medullaris* (Bower, 1923, vol. i, p. 279) has yielded the following measurements, the area being taken as the square of the radius:

	Stem.	Cortex.	Stele.	Pith.
Radius (mm.)	65	12	53	47
Cross-sectional area (mm. ²)	4225	1416	2809	2209
$\frac{\text{Radius cortex}}{\text{Radius pith}} = 0.26$		$\frac{\text{Area cortex}}{\text{Area pith}} = 0.64$		

In this species the pith is a very conspicuous feature. It is interesting to note that the medulla is traversed by numerous small accessory vascular strands. In *Cibotium Barometz* the pith may enlarge very considerably without

bringing about a disruption of the solenostele, its cross-sectional area being actually greater than that of the cortex. In *Hemitelia setosa* with a dictyostelic vascular system and accessory medullary strands the ratio $Ac : Ap$ is 1.3 and in *Dicksonia antarctica* 1.2.

Dryopteris aristata

At the point of departure of the slender rhizome from an erect shoot the stele is a simple protostele. On further growth a central pith develops, soon

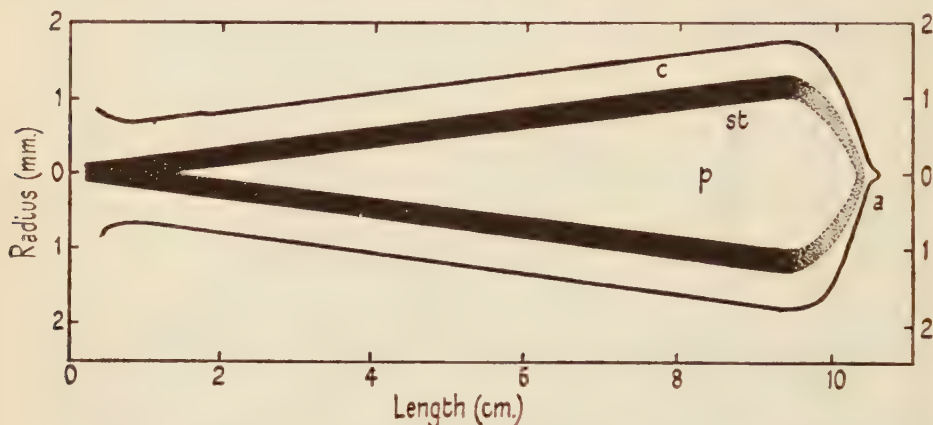


FIG. 17. *Dryopteris aristata*. Diagrammatic representation of a horizontal rhizome (leaves, leaf-gaps, and roots omitted) to scale, showing the progressive development of pith (p), stele (st), and cortex (c); apex (a).

to be followed by a conspicuous enlargement of the stele. A solenostelic and a dictyostelic condition are to be observed at the levels of departure of the first and second scale leaves respectively. The gradual increase in diameter which can usually be observed in the underground rhizome, with its widely separated fleshy scale leaves, is replaced by a marked increase when the rhizome develops into an erect or semi-erect shoot with closely spaced foliage leaves. As rhizomes up to 20 cm. in length, possessing no assimilating leaves, have on occasion been found in humus, the translocation of the very considerable quantities of organic substances required for this growth from the parent shoot, by way of the small proximal protostele, may be considered a remarkable phenomenon and one which raises many interesting questions.

Fig. 17 shows in diagrammatic form (to scale) the relations of pith, stele, and cortex in a rhizome which has eventually developed into an erect shoot, the widening of the pith being a conspicuous feature. Various ratios are indicated in Fig. 18. The ratios *radius stem* to *radius stele* and *radius cortex* to *radius pith* both decrease in a conspicuous manner during development but increase again in the region of the apex. A conspicuous change in the ratio of cortex to pith takes place as the rhizome increases in diameter. Thus, at the solenostelic level near the base of the rhizome, Fig. 18, the ratio $Ac : Ap$

is 2.1, whereas it falls to 2.6 in the thickest region of the rhizome and to 1.6 in the stout erect shoot. Data relating to the development of pith and cortex in the region behind the terminal meristem of the erect shoot have also been

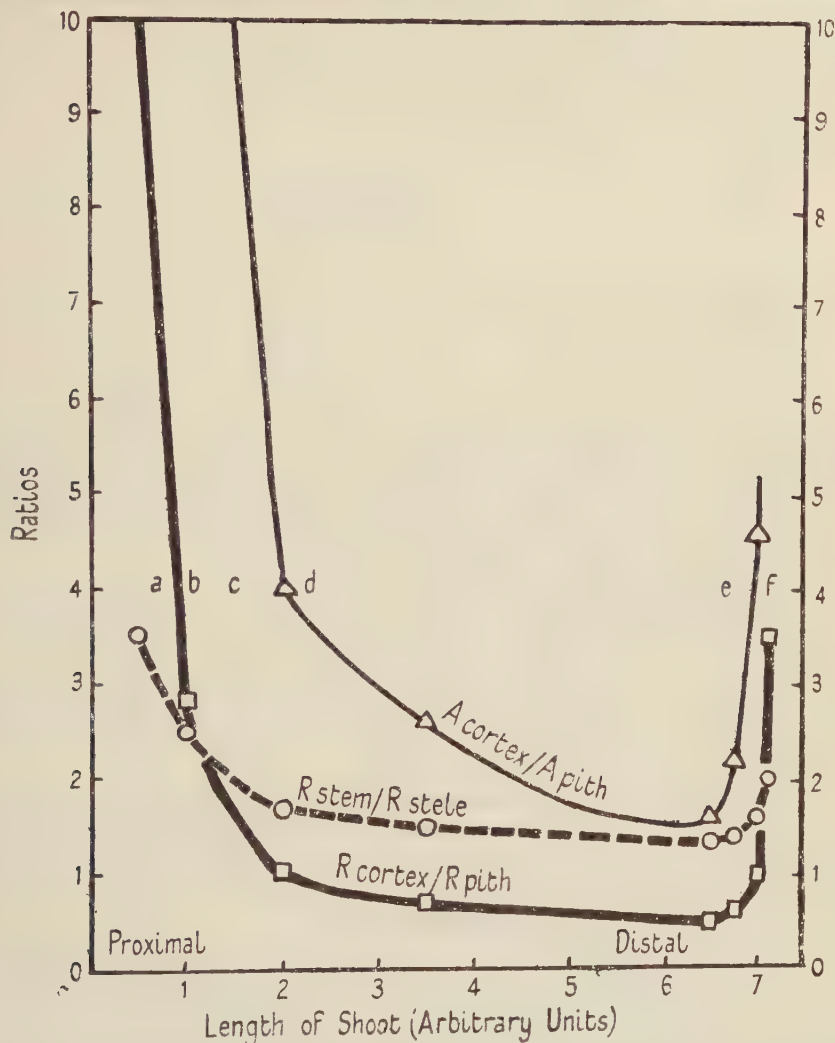


FIG. 18. *Dryopteris aristata*. Curves showing ratios relating to the pith, stele, and cortex during the development of a lateral rhizome. R, radius; A, cross-sectional area. a, solid protosteles; b, medullated protosteles; c, solenosteles; d, dictyosteles; e, thickest region of fully differentiated shoot; f, apical region.

incorporated in Figs. 17 and 18. (The data are those obtained by measuring the dimensions of stem, cortex, &c., in a plane at right angles to the long axis of the shoot. Certain inaccuracies are thereby involved, as they also are in other systems of measurement which have been attempted. They suffice,

however, to give approximate values for the relative growth developments under consideration.) In the basipetally enlarging apical cone the amount of parenchymatous development in the cortex is greater than in the pith, but lower down, as the stem enlarges to its maximum diameter, the ratio $Ac:Ap$ gradually diminishes to a value of 1.5, i.e. slightly lower than that observed in the mature region of the shoot lower down.

In a rhizome of *D. aristata* the following measurements were made, the area being taken as the square of the radius:

	Cross-sectional area (mm. ²)		$\frac{Ac}{Ap}$	$\frac{E.S.}{I.S.}$
	Cortex.	Pith.		
(a) Continuous vascular ring at apex	0.052	0.026	2.0	1.8
(b) Differentiated dictyostele lower down	0.90	0.53	1.7	1.2

The dimensions of the external and internal stelar surfaces, through which the outward and inward diffusion of nutrients may take place, were also compared. The ratios *external surface (E.S.)* to *internal surface (I.S.)* were 1.8 for (a) and 1.2 for (b). In other words, as the pith enlarges the dimensions of the external and internal stelar surfaces approximate more closely.

The following data relate to the same rhizome: section (a), cross-sectional area of undifferentiated vascular ring at apex = 0.058 mm.²; section (b) cross-sectional area of differentiated meristeles lower down = 0.087 mm.² The ratio b/a is thus 1.5, that for the cross-sectional areas of pith, 20.4, and that for the pith radii is 4.6. Thus during development in the region below the apical meristem the rate of growth in the transverse plane of the pith parenchyma greatly exceeds that of the vascular tissue.

A comparison of the relative developments of pith and cortex at levels progressively farther away from the apex (Fig. 18) indicates that the growth of cortical tissue gradually slows down while that of the pith is still active, till finally in the adult region of large shoots the cross-sectional area of the pith does not fall far short of that of the cortex.

The sections of leaf primordia in Figs. 9–13 are roughly semicircular in outline. An approximately representative series of measurements can therefore be obtained by regarding a line drawn in the direction $a\ b$ (Fig. 13) as a diameter. On this basis the ratios shown in Fig. 19 have been obtained. It will be seen that these correspond closely with those shown on the right-hand side of Fig. 18, the ratio *radius cortex* to *radius pith* falling to 0.6 in the older petioles (0.5 was observed in the shoot), and the ratio $Ac:Ap$ falling to 1.7 (1.6 in the shoot). In a large petiole base of *D. filix-mas* the ratio *radius cortex* to *radius pith* was 0.34, and $Ac:Ap$, 0.94. In *Nephrolepis cordifolia* tubers the corresponding ratios were 0.5 and 1.26 respectively. Such data give a clear indication of the importance of medullation in the internal morphology of the leaf-base in species of Dryopteris.

Dryopteris filix-mas

In general, data for this species are comparable with those cited above for *D. aristata*. Figs. 20-6 show the protostelic, solenostelic, and dictyostelic condition at the base of a thin rhizomatous plant derived from a lateral bud,

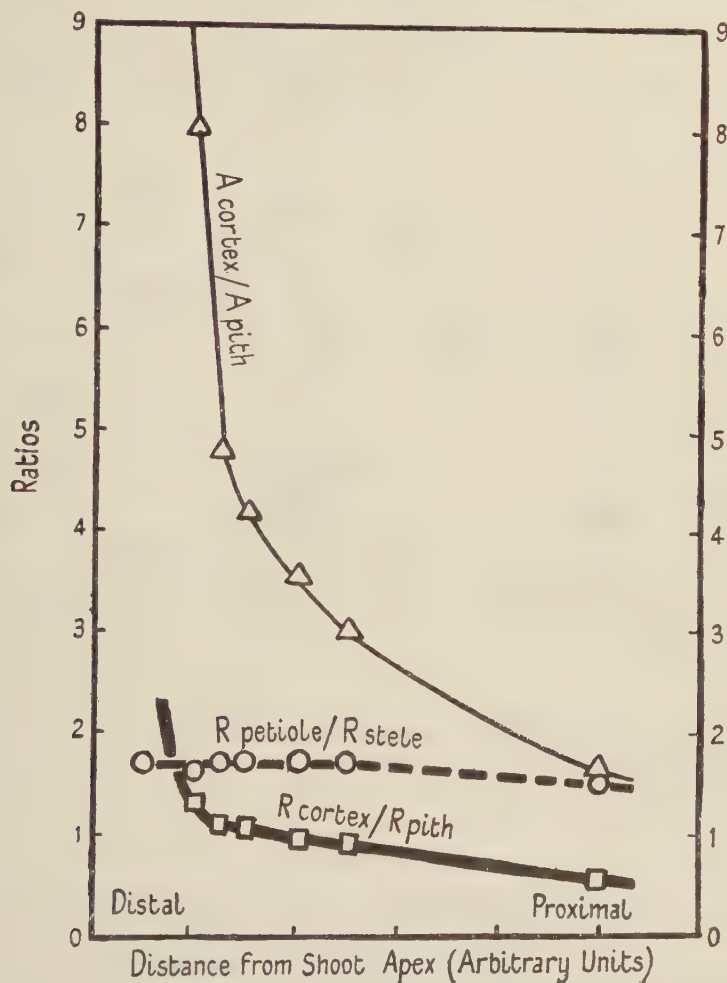
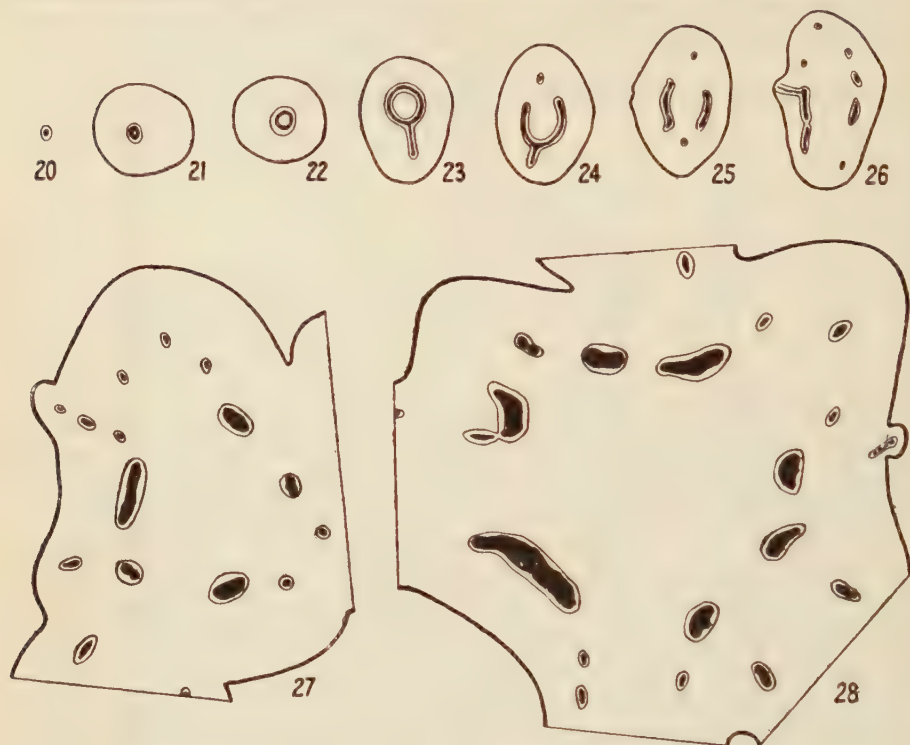


FIG. 19. *Dryopteris aristata*. Curves showing ratios relating to the pith, stele, and cortex of leaf primordia during development (data from Figs. 9-14, &c.). R , radius; A , cross-sectional area. These data correspond to those on the right-hand side of Fig. 12.

the relatively small cross-sectional areas of shoot and leaf-bases being apparent. By contrast, Figs. 27 and 28 show the relatively large cross-sectional area of the erect, bulky, leafy shoot. It is characteristic of this species that the bases of foliage leaves become so distended as to be almost circular in cross-section,

the enlargement of the pith and the subdivision of the vascular system into seven or more strands being conspicuous features. The several ratios under consideration show the same general trends as do those for *D. aristata*.



FIGS. 20-8. *Dryopteris filix-mas*. Figs. 20-6 protostelic, solenostelic, and dictyostelic conditions observed in acropetal succession from the proximal end in a thin underground rhizome. Fig. 20. Initial small stele of petiole bud. Figs. 27 and 28. Sections through two erect shoots of different sizes. ($\times 5$.)

DISCUSSION

In leptosporangiate ferns remarkable changes in internal structure may be observed throughout the development of the individual plant from the sporeling and also in the region immediately below the apical meristem in the adult plant. The vascular tissue and the several parenchymatous regions of the leafy shoot are all differentiated more or less contemporaneously in the growing region. The rates of growth of these tissues are, however, initially different and change during development. Hence, in any causal account of the development of the internal pattern of the adult leafy shoot, the system of relations obtaining in the apical region must be investigated as a dynamic whole. In the complex pattern of the adult petiole or shoot the pith, which is absent in the sporeling, is seen to be an important component.

In the tubers in *Nephrolepis cordifolia*, the swollen leaf-bases in *Dryopteris*

felix-mas, and in certain large solenostelic and dictyostelic shoots, the amount of parenchymatous development in cortex and pith is approximately equal. Such observations raise the question, as yet unsolved, as to the manner in which metabolites are supplied to the growing region, i.e. by diffusion outwards and inwards from the vascular tissue, or by diffusion from below over the whole cross-sectional area. The ratio cross-sectional area cortex to that of pith which is high in the sporeling of leptosporangiate ferns tends to approach unity in the adult shoot.

During the individual development in leptosporangiate ferns the solid protostele present in the young sporeling becomes medullated, i.e. thin-walled parenchyma develops in the central region of the cylinder of tracheides. It has been argued (Bower, 1923, where the earlier literature is summarized) that such medullation represents a 'change of destination' of elements initially and potentially tracheidal, and that phylogenetically the origin of the pith is to be sought in a loss of conducting function in the central core of tracheides. The argument is that with increase in size and with water being mainly withdrawn from the more peripherally situated tracheides, there would tend to be a stagnation of water in the more central tracheides; hence their conductive function would eventually disappear and their place be taken by a water-storing pith parenchyma. Support is given to this hypothesis by the occasional occurrence of pteridophytes possessing a 'mixed pith', i.e. with tracheidal elements scattered among the cells of the pith parenchyma. A somewhat different view of these histological developments is taken here; for even if it be accepted that phylogenetically the pith represents modified tracheidal tissue, its development in the individual solenostelic or dictyostelic species must still be accounted for in terms of the processes of growth at the apex. This needs thorough investigation if any adequate account of the internal morphology of the shoot is to be given.

In the development of the sporeling the first appearance of the pith marks a critical phase in the morphology of the stele. The data submitted by Bower (1930) suggest that in the individual species the size-factor may be involved, but whether the increase in size and the concomitant modification in the stele are to be referred to mechanical or physiological factors, or to a combination of both, is not known. The data submitted here suggest that the mode of distribution of metabolites and the development of mechanical stresses in the formative region may be factors in the situation.

The account given by Tansley (1907) of the development of the leaf-gap in solenostelic ferns relates to physiological conditions which he considers may obtain in the developing stele. In his view, the transpiration current which is partly deflected into the leaf-trace 'will take a peripheral course and the tracheidal elements of the stele opposite the middle of the trace will tend to be depleted of water and eventually cease to be developed'. The evidence now presented suggests that the phenomenon under consideration should be interpreted in terms of the process of growth and not of functional activities associated with later stages of differentiation. Tansley also considered that

the formation of a leaf-gap was 'a necessary consequence of the great dilation of the stele, and the concomitant thinning of the vascular ring, involved in the formation of a solenostele'. The present writer (1944a) has shown that the leaf-gap develops in direct relation to the enlargement of the vascular system of the leaf-base. The part played by the enlarging pith in causing a stelar disintegration is, however, clearly seen in the vascular system of petioles of *Dryopteris* and other ferns and in tubers of *Nephrolepis cordifolia*.

In dicotyledons, Kostytschew (1922) and Priestley (1928) have recognized that the procambial strands take their origin basipetally from a continuous ring of distinctive tissue which underlies the apical meristem, 'the subsequent isolation into bundles occurring through development of parenchymatous tissue from part of the ring when in other parts of the ring vascular elements differentiate in connection with the bases of the leaf initials'. The general resemblance to conditions in leptosporangiate ferns is evident; moreover, Priestley and Scott (1937) have shown in *Tradescantia* and *Alstromeria* that the apparently confluent or 'closed' system of vascular strands in the leaf can be referred to the development of panels of parenchyma within the original coherent and unbroken procambial tissue present in the juvenile stage.

A survey of the facts of development, some of which have been dealt with here, makes it unnecessary to consider in detail conceptions such as that of Jeffreys (1903) in which the pith is regarded as being due to an intrusion of cortical tissue into the stele.

Hitherto studies of translocation have been chiefly devoted to the movement of material in fully differentiated vascular tissues, specific functions being allotted to the phloem and xylem. But at the apical growing-point, both in the regions of organ formation and of enlargement, the vascular system is still seen to be in the initial phase of differentiation, i.e. it consists of small-celled tissue, distinct from the parenchyma of cortex and pith, but showing no differentiation into phloem and xylem. The extent to which this tissue serves to conduct the metabolites necessary for the maintenance of the actively developing region has received little attention at the hands of physiologists. Whatever the functional activity of this undifferentiated vascular tissue may be, it is important to note that the characteristic morphology of the adult shoot is already established in the subterminal region, Figs. 6, 7, and 8. The observations set out indicate that the changing dimensions of the pith in leaf-base and shoot are closely related to the changing configuration of the vascular system and that medullation must be taken into account in considering the operation of the size-factor in stelar morphology.

SUMMARY

1. An account is given of the development of the vascular system in ferns from its point of origin immediately below the apical meristem, special attention being paid to medullation and the formation of the leaf-gaps.

2. An examination of the shoot apex throughout development from the sporeling to the adult shows that the histological character of the newly

differentiated vascular tissue remains constant; this is in marked contrast to the structural diversity shown by the fully differentiated stele at different levels in the shoot.

3. The origin of leaf-gaps has been related to the enlargement and disintegration of the vascular system in the leaf-bases, differences in the rates of growth of pith and vascular tissue and the development of mechanical stress in the latter being factors in the situation.

4. The relative developments of cortex and pith as they affect the internal morphology are examined in shoots, leaf-bases, and tubers at different stages of development. The ratio *cross-sectional area of cortex* to *cross-sectional area of pith* decreases in a notable manner as organs increase in size.

5. These observations are discussed in relation to earlier views on factors affecting stelar morphology. It is suggested that the various structural developments, which are to be observed immediately below the apical meristem, should be interpreted in terms of the process of growth and not of the functional activities associated with later stages of differentiation.

The writer is much indebted to Mr. E. Ashby for assistance in the preparation of materials.

LITERATURE CITED

- BOWER, F. O., 1923: *The Ferns*. Vol. I. Cambridge.
 — 1930: *Size and Form in Plants*. London.
 JEFFREY, E. C., 1903: The Structure and Development of the Stem in Pteridophyta and Gymnosperms. *Phil. Trans. Roy. Soc. Lond.*, cxcv. 119.
 KOSTYTSCHEW, S., 1922: Der Bau und das Dickenwachstum der Dikotylenstämme. *Ber. der deut. bot. Ges.*, xl. 297.
 PRIESTLEY, J. H., 1928: The Meristematic Tissues of the Plant. *Cambr. Phil. Soc. Biol. Rev.*, iii. 1.
 — and SCOTT, L. I., 1937: Leaf Venation and Leaf Trace in the Monocotyledons. *Proc. Leeds Phil. Soc.*, iii. 305.
 TANSLEY, A. G., 1907: *Lectures on the Evolution of the Filicinean Vascular System*. New Phyt., vi. 150.
 WARDLAW, C. W., 1944: Experimental and Analytical Studies of Pteridophytes. III. Stelar Morphology. The Initial Differentiation of Vascular Tissue. *Ann. Bot., N.S.*, viii. 173.
 —, 1944a: Experimental and Analytical Studies of Pteridophytes. IV. Stelar Morphology: Experimental Observations on the Relation between Leaf Development and Stelar Morphology in Species of *Dryopteris* and *Onoclea*. *Ann. Bot., N.S.*, viii. 387.

Studies in the Vernalisation of Cereals

IX. Auxin Production during Development and Ripening of the Anther and Carpel of Spring and Winter Rye

BY

E. S. J. HATCHER

(*Research Institute of Plant Physiology, Imperial College of Science and Technology, London*)

With four Figures in the Text

	PAGE
INTRODUCTION	235
EXPERIMENTAL METHODS	236
Determination of auxin content	236
Extraction of auxin	238
AUXIN CONTENT OF MATURE GRAIN	239
Diffusion experiments	239
Extraction experiments	240
Distribution of auxin in mature grain	242
AUXIN CONTENT OF GRAIN DURING DEVELOPMENT	243
1941 experiments	243
1942 experiments	244
1943 experiments	248
AUXIN CONTENT OF PREMATURELY HARVESTED GRAIN	251
AUXIN CONTENT OF THE EAR DURING DEVELOPMENT	257
DISCUSSION	258
Nature of auxin production in the developing grain	258
Auxin systems of the grain	260
Nature of the precursor	262
The function of the auxins in development	262
SUMMARY	263
LITERATURE CITED	265

INTRODUCTION

EARLIER papers in this series have been concerned with the problem of vernalisation with special reference to rye. The possibility of a hormone mechanism underlying the vernalising process was earlier recognized (Purvis and Gregory, 1937; Gregory and Purvis, 1938) and a cytological study of the developing rye grain (Nutman, 1939) brought forward evidence of hormonal activity. A systematic study of the hormone relations of the grain was therefore projected, and this paper embodies the results of an investigation on the auxin complex. It will appear later that the auxin relations afford no clue to the specific vernalising effect, but a critical examination has been possible of the hypothesis of auxin control of vernalisation put forward by Cholodny (1936). His hypothesis was based principally on auxin studies of germinating grains (Cholodny, 1935) which indicated that auxin is present in the endosperm, and further that there is a disappearance of auxin during germination.

[*Annals of Botany*, N.S. Vol. IX, No. 35, July, 1945.]

In essence the hypothesis is that during vernalisation, when growth is almost at a standstill, auxin from the endosperm reaches an abnormally high concentration in the embryo, whereas at normal temperature it is expended in growth. The effect of this, according to Cholodny, is to accelerate the passage of the tissues through the developmental phases preceding the initiation of the flower as is characteristic of vernalisation. Cholodny does not appear to have put his hypothesis to any stringent experimental test, and it has in consequence always lacked support of a direct nature.

Available evidence for rye is quite contrary to Cholodny's hypothesis. First, the excised embryo can be vernalised independently of the endosperm and the auxin it contains (Gregory and Purvis, 1938). The objection raised by Cholodny (1939) on the ground of preliminary soaking during superficial sterilization has been met by the experiment of Purvis (1944) in which dry excised embryos have been successfully vernalised, and thus in complete independence of the auxin stored in the endosperm. Second, the developmental rate up to flower initiation, as measured by leaf production is the same in vernalised and control plants (Purvis, 1934). Further, embryos can be vernalised during their development in the ear (Gregory and Purvis, 1936, 1938), which fact led to a thorough comparative study of the developing grain (Nutman, 1939) with the result that no striking dissimilarity between spring and winter varieties of rye was observed to account for the contrasting behaviour of the plants grown from them. However, it was this study that produced evidence for the occurrence in the carpel of growth-promoting substances, and led to a direct study of auxin in the grain. Some of the more important results have already been briefly reported (Hatcher and Gregory, 1941; Hatcher, 1943) and are here presented in their entirety.

Before proceeding to a description of the experiments mention must be made of some data of Thimann and Lane (1938) considered by them, and by Cholodny (1939), to support the auxin hypothesis. Thimann and Lane noted a marked shortening of the first leaf after the low-temperature treatment necessary for vernalisation, and suggested this as a possible diagnostic character of the stage of vernalisation reached. It has since been shown by the author that this is not specifically related to vernalisation at all since similar results have been obtained with spring and winter varieties of rye, of which the spring form shows no vernalising effect of low temperature so characteristic of the winter form. This shortening of the leaf is a direct effect of low temperature; nor is it the sole morphological change, since later work has shown that the development of epidermal hairs on the bases of the early leaves is also controlled by low temperature during germination in both varieties. These findings will be discussed at length in a later communication.

EXPERIMENTAL METHODS

Determination of auxin content.

Auxins occur in such minute traces that only bio-assay methods are practicable for their estimation, and of several which have been developed the one

used most extensively is the *Avena* method of Went (1928). Even this has variants, such as the deseeded method of Skoog (1937), but in the present study the standard technique fully described by Went and Thimann (1937) has been adopted, using for the purpose an underground chamber controlled at 25° C. and 85 per cent. relative humidity.

The *Avena* variety employed was the Svalöf (Sweden) strain of 'Victory', a line of which has been maintained in recent years by the Institute.

The interval between the two decapitations of the coleoptile was 3 hours, and shadowgraph records were taken when the agar blocks had been in contact with the decapitated stump for 1½ hours. The blocks, each 10 c.mm. in volume, were cut from plates of 3 per cent. agar into which were first introduced the substances under estimation. For each assay 8 coleoptiles were used; shadowgraph curvatures were measured to the nearest degree, and mean curvatures of 2° or more were accepted as proof of the presence of auxin. Several series were run for each estimation, with varying concentrations so as to secure one falling within the appropriate proportionality range.

Auxin was introduced into the agar plates either by direct diffusion from a freshly cut surface of the plant material or by immersion in an aqueous extract. In the first method diffusion took place for 2 hours in a saturated atmosphere to prevent drying out of the agar and plant tissues. After removal of the plant material the plate was left for ¾-hour before cutting into blocks to ensure uniform distribution of the diffusates. Results were calculated as shown below, following the method of Avery et al. (1941).

$$\begin{aligned}\text{Total degrees curvature} &= \text{Mean curvature} \times \text{number of blocks per plate.} \\ \text{T.D.C.} &= C^\circ \times 12.\end{aligned}$$

This method estimates the relative amount of auxin present, but does not allow estimation of total auxin in the tissue, since diffusion from the plant material is not completed in 2 hours.

In the second method the agar plate was immersed for 2 hours in an aqueous extract, after which time the concentration of dissolved substances was uniform throughout solution and agar. This enabled the blocks to be cut immediately after pouring off the solution. So long as the volume of the solution does not fall below 2 c.c., the addition of the agar plate does not affect the concentration sufficiently to necessitate a correction. Results were calculated as follows:

$$\begin{aligned}\text{Total degrees curvature} &= \text{Mean curvature} \times \text{volume of solution (c.c.)} \times \\ &\quad \text{number of blocks equal in volume to 1 c.c.} \\ \text{T.D.C.} &= C^\circ \times V \text{ c.c.} \times 100.\end{aligned}$$

With standard solutions of indole-3-acetic acid the concentrations giving curvatures within the proportionality range are from 25 to 150 µg. per litre (1,000 µg. per litre = 1 mg. per litre = 1 part per million). This provides a valuable comparison in considering results expressed as T.D.C., for 1 µg. indole-3-acetic acid is equivalent to roughly 30,000 T.D.C. In expressing results it has been found convenient to use 1,000 T.D.C. as the unit.

Extraction of auxin.

When the present investigations were begun in 1940 information on extraction technique was insufficient to justify without further tests the adoption of any method then in use, and accordingly preliminary experiments were made to determine a suitable procedure.

Went (1928) had attempted, without success, a water extraction of crushed coleoptile tips. Thimann (1934) using acidulated chloroform obtained active extracts from coleoptiles, and also identified auxin in wheat germ, *Vicia* buds, and *Sequoia* pollen. Van Overbeek (1938) used ether as a solvent, without acid present, and his results failed to support Thimann's contention that an acid medium was requisite. Meanwhile Laibach and Meyer (1935) had extracted auxin with hot acidulated alcohol from *Zea* and *Helianthus* seeds and seedlings. It appeared that in the extraction of auxin from seedlings precautions had to be taken against the destruction of auxin by oxidative enzymes, so that the use of water as a solvent was not recommended. A further precaution was taken by du Buy (1938), who first froze the material with solid CO₂, a method now frequently employed in the ether extraction of auxin from growing tissues. So far as dry seed material is concerned, however, quite the contrary result was obtained, for water extraction gave the highest yield of auxin. Cholodny (1935) first reported this, stating that whereas alcohol failed to extract auxin from oat grains, water gave good yields, while water-alcohol mixtures gave intermediate values varying with the proportion of water used. The present work shows that alcohol does in fact extract auxin from oat grains, which Cholodny's assay method (external application to intact coleoptiles) was not sensitive enough to detect. Again van Overbeek, while using ether for coleoptiles (1938), used water for extracting maize grains (1938 *a*); and Avery having first developed and recommended an alcohol method for maize grains (1939) subsequently found water to be superior as a solvent (Avery et al. 1940). The same result was also obtained in the preliminary experiments of this investigation, and will be considered in the section dealing with the auxin content of mature grain. It was therefore decided to use water as the solvent in the developmental studies of the grain.

The practical details of the methods employed are relatively simple, and will be described here quite briefly so that in reference later to any particular experiment it will be sufficient to state the method employed.

(i) *Organic solvents*, e.g. ether, chloroform, alcohol. These were used only in the preliminary experiments with dry grains, which were ground with sand using pestle and mortar, either alone or in the presence of the solvent. The extraction time varied from a few minutes to several days, and even weeks. After filtration from the material the extract was evaporated to dryness, the residue dissolved in a small volume of water, and from this solution dilutions of different strength were made up and assayed for auxin.

(ii *a*) *Water extraction of dry grains*. The sample of grains was sometimes ground dry with pestle and mortar, or in a mill, sometimes in the presence of water. The extraction period was for 10 minutes in a water-bath at 80° C.

or boiling. The extract was separated from the ground material by suction filtering or by centrifuging. The filtrate or centrifugate was evaporated to small volume at reduced pressure, and from the concentrate dilutions made up for assay. At first the original extract was not tested for auxin before concentration, but later this was always done.

(ii *b*) *Water extraction of developing grains or other watery material.* The sample was ground with sand in a known volume of water. The extraction period was for 10 minutes in a water-bath at 80° C. or boiling, though the flask containing the extract could be left overnight before heating. The extract was filtered or centrifuged and further procedure was as for dry grain.

(iii) *Alkaline hydrolysis.* The introduction of an alkaline heat treatment will be discussed later. The procedure was similar to that with water, except that after alkaline treatment the pH of the extract was adjusted before assay.

AUXIN CONTENT OF MATURE GRAIN

The main study was with Spring and Winter forms of Petkus rye, but all the common cereal grains have been examined: maize, oat, wheat, barley, and rye. It is unnecessary to enumerate every experiment performed. The early experiments were exploratory in nature, and the work, being on dry grain, was done at various times during the last three winters. The results are presented in the form of a comprehensive summary.

TABLE I

Auxin Content of Mature Grain (1,000 T.D.C. units)

Cereal	Maize.	Oat.	Rye.	Wheat.	Barley.
<i>Diffusion method</i>					
(i) Embryo section	0.138	0.074	<0.004	<0.008	<0.006
(ii) Distal section	0.076	0.034			
<i>Extraction method</i>					
(i) Ether	4.04	—	<0.016	—	—
(ii) Chloroform	—	—	<0.016	—	—
(iii) Alcohol	3.19	0.075	<0.008	—	—
(iv) Water	9.85	0.280	0.062	0.042	0.056
<i>Alkaline hydrolysis</i>					
(i) pH 10 buffer	121.7	4.68	2.80	—	—
(ii) N/50 NaOH	234.2	7.48	5.93	3.92	1.45
<i>Auxin ratios</i>					
N/50 NaOH : water	24.00	27.00	96.00	>93.00	>26.00
<i>Concentration total auxin</i>					
(auxin per mg.)	0.820	0.237	0.148	0.102	0.029
<i>Indole-3-acetic acid</i>					
equivalents	7.81	0.25	0.20	0.13	0.05
(μ g per grain)					

Many of the entries in this table are the means of several estimations.

Diffusion experiments.

With the standard diffusion method only maize and oat have given positive results, though with prolonged diffusion into agar, 24 hours as against the

2 hours standard time, slight curvatures were obtained with rye. Much more auxin diffused from the maize than from the oat grain, and in both cases the auxin from the half of the grain containing the embryo exceeded that from the other section. The failure of Cholodny (1935) to obtain a detectable amount of diffusible auxin from oat must be attributed to the less sensitive method of assay he employed (see above). The results in units of 1,000 T.D.C. are entered in Table I. It may be concluded that auxin content is highest in the maize grain, intermediate in oat, and too small to be detected by diffusion in rye, barley, and wheat.

Extraction experiments.

Using absolute alcohol, only maize and oat gave positive results, and in each case successive extractions for long periods of time yielded further auxin, indicating that total extraction with this solvent cannot be effected rapidly. This suggests the possibility that a gradual release of auxin occurs during extraction. Serial extractions of rye grains using ether and chloroform failed as with alcohol to give curvatures; which does not necessarily imply the total absence in rye of auxin extractable by these solvents, but that if present the quantities fall below the level of estimation.

With water, auxin has been successfully extracted from all the cereals, and always in higher quantities than with organic solvents. Yields tend to be variable, in rye for instance ranging from 0.04 to over 0.10 units. After exhaustive extraction with an organic solvent considerable auxin is still extracted in water: thus with a 500-grain sample of rye three successive alcohol extractions failed to give any curvatures, i.e. less than 0.002 units per grain, while subsequent extraction with water gave an auxin yield of 0.04 units per grain. Using water-alcohol mixtures Cholodny's results (1935) were confirmed, the higher the proportion of water, the greater the amount of auxin extracted, as the following results with rye in Table II show:

TABLE II
Extraction of Auxin by Water-Alcohol Mixtures

Vol. of water.	Vol. of alcohol.	Auxin units (1,000 T.D.C.)
0	30	less than 0.008
5	25	0.014
15	15	0.022
25	5	0.032
30	0	0.040

Cholodny concluded that free auxin is formed immediately on the addition of water, but though this may be so, the successful extraction of auxin by organic solvents from maize and oat discounts the implication that no free auxin is present in the dry grain. The bearing of these results on the question of the state of auxin in the grain, however, will be considered later.

At this point it is opportune to consider the comprehensive investigations

of Avery et al. (1940), using mature maize endosperm, to compare all the extraction methods previously employed by other workers. Maize, as has been seen, is the cereal grain richest in auxin, and therefore is eminently suited to such a study. Of the different methods compared Avery et al. found that water extraction gave the highest yields, but that a multi-solvent method appeared to be more effective than extraction by any single solvent. Accordingly water extraction and multi-solvent extraction were studied further (Avery et al., 1941), and it was shown conclusively that neither alcohol nor chloroform alone extracted as much as when used successively, and that water following alcohol and chloroform extracted yet more auxin. Using water as the first solvent in a multi-solvent method, however, almost all the auxin was extracted with serial washings with water, and very little by the others; so that repeated water extraction gives almost the same yields as multi-solvent extraction. It was further noted that duration of extraction was an important factor which suggested the release of auxin during extraction by some process of hydrolysis. Accordingly the effects of duration, temperature, and pH or auxin yield were investigated, and the important result established that alkaline conditions, optimal at pH 10, slowly at room temperature, and within a few minutes at 100° C., released a large quantity of free auxin from an inactive precursor present, thereby increasing the yield of auxin by approximately 10 times. The precursor is soluble in water, and insoluble in organic solvents,¹ as was shown by alkaline hydrolysis of the separated extracts. In water extract, at pH 7, there was found to be a slow release from the precursor, and this possibly accounts for the somewhat variable auxin content of water extracts of cereal grains mentioned above (p. 240).

These findings of Avery were immediately applied in the present studies of rye, and late in 1941 the effect of alkaline hydrolysis was confirmed; a phosphate buffer of pH 10 increased the yield of extractable auxin some 20 times, and all the other cereal grains examined responded in the same way to alkaline treatment. Subsequently the optimal conditions for alkaline hydrolysis in rye were determined. By extracting rye flour with varying concentrations of sodium hydroxide solution, N/50 NaOH was found to give a yield of auxin about double that given by phosphate buffer of pH 10. The results with sodium hydroxide are entered in Table III. The cereal grain thus

TABLE III

Optimal Conditions for Release of Auxin from Rye Wholemeal.
(Auxin yields expressed per gramme in 1,000 T.D.C. units)

NaOH solution	N/500.	N/200.	N/100.	N/50.	N/25.	N/10.
Auxin yield	57.6	98.0	131.6	153.6	146.3	106.9
				144.0	—	92.9
				151.2		
Auxin yield with						
water	—	3.7				
pH 10 buffer	—	74.4				

¹ It has since been shown by Berger and Avery (1944 a) that the precursor of maize is extractable in 50 per cent. acetone-water solution.

contains some free auxin extractable by organic solvents and water, and a much greater quantity of an inactive substance from which alkaline hydrolysis releases auxin. The released auxin is destroyed by acid treatment suggesting indole-3-acetic acid as the active substance, as against auxins *a* and *b* which are not stable in alkaline conditions (Went and Thimann, 1937).

All the foregoing results with mature grains of cereals are summarized in Table I. The cereals vary considerably both in the auxin content per grain and the concentration of auxin as expressed in units of 1,000 T.D.C. per mg.; the comparative efficiencies of organic solvents, water, and alkali as extracting agents for auxin are similar for all of them.

Distribution of auxin in mature grain.

The diffusion experiments showed that auxin tends to be concentrated in the proximity of the embryo (maize and oat), and the investigation was extended to locate more precisely the storage region. First rye embryos were excised and extracted separately with and without alkaline treatment which established that auxin was not present in detectable amount, for less than 0.027 unit of auxin was present in the embryo as compared with 3.37 units extractable from the total grain by alkaline hydrolysis using the phosphate pH 10 buffer. This indicated that not more than $\frac{1}{2}$ per cent. of the auxin could occur in the embryo, although the embryo constitutes $2\frac{1}{2}$ –3 per cent. of the weight of the grain; some 99 $\frac{1}{2}$ per cent. of the total auxin is thus located in the aleurone and endosperm tissues.

TABLE IV

*Location of Auxin in the Rye Grain.
Extraction with pH 10 buffer (1,000 T.D.C. units)*

Part of grain extracted.	Mean weight (mg.).	Auxin content (1,000 T.D.C. units).	Auxin concentration.	Percentage of total auxin.
Experiment 1.				
Embryo section	11.0	2.31	0.210	53
Middle section	18.4	1.11	0.060	25
Distal section	14.4	0.96	0.067	22
Total grain	43.8	4.38	0.100	100
Experiment 2.				
Embryo section	9.6	1.42	0.148	55
Middle section	16.5	0.67	0.041	26
Distal section	11.4	0.50	0.044	19
Total grain	37.5	2.59	0.084	100

In Table IV are given the results of extracting separately three portions of the grain, namely embryo section, middle section, and distal section. Evidently over 50 per cent. of the auxin is present in the embryo section (but not in the embryo itself), and since this constitutes roughly a quarter of the total weight of the grain the auxin concentration in this region is some $3\frac{1}{2}$ times that in the rest of the grain.

The rye grain is characterized by a blue aleurone layer which can be scraped away fairly easily. Such aleurone scrapings (including the pericarp), constituting about a quarter of the total grain weight, were extracted separately from the endosperm and nearly three-quarters of the total auxin was found to be in the scrapings, the concentration of auxin in the aleurone thus being 8 times that in the endosperm proper, as shown by the accompanying data in Table V:

TABLE V

Distribution of Auxin in Aleurone and Endosperm of Rye Grain
(1,000 T.D.C. units)

Region of grain.	Weight (mg.).	Auxin content.	Percentage auxin.	Auxin conc. (per mg.).
Aleurone+pericarp . . .	9.6	2.34	71	0.244
Endosperm . . .	29.6	0.95	29	0.032
Total . . .	39.2	3.29	100	0.084

It may be concluded therefore that the auxin of the rye grain is found chiefly in the aleurone layer, the major portion being near the embryo.

AUXIN CONTENT OF GRAIN DURING DEVELOPMENT

Studies were made during the summers of 1941, 1942, and 1943. In 1941 diffusion and water extraction were employed; in 1942 water extraction and alkaline hydrolysis were systematically compared using a pH 10 phosphate buffer for the hydrolysis; in 1943 the methods comprised water extraction, alkaline hydrolysis using N/50 NaOH (the optimal concentration), alkaline hydrolysis using an unbuffered NaOH solution of pH 10, and alkaline hydrolysis using N/10 NaOH solution.

1941 experiments.

A series of auxin estimations was made on each of the following sets of plants: (1) winter rye, var. Petkus, autumn sown, anthesis date June 16-19; (2) spring rye, var. Petkus, spring sown, anthesis date June 27-30; (3) winter rye, var. Petkus, vernalised for varying periods in spring; sand cultured; anthesis over period July 10 to August 9.

Individual ears were labelled on the day the first anthers protruded. The normal time from anthesis to harvest in good weather is 6 weeks, though ripening is always liable to be delayed by wet weather. The autumn sown winter rye plants were ripe at 6 weeks, when they were harvested, but the spring rye plants were exposed to indifferent weather conditions a month after anthesis, and were not ready for harvest until the eighth week. The vernalised winter rye plants were also as a rule not ripe for harvest at 6 weeks.

In the first series at weekly intervals water extracts of developing grain were made and estimated for auxin. The practice was first to concentrate the extract at reduced pressure, and then to make up a series of dilutions. The results for this series, as well as for the second and third, are summarized in Table VI.

TABLE VI

Auxin Content of Developing Grain. 1941 Experiments.
(1,000 T.D.C. units)

Weeks from anthesis.	Series 1 Winter rye.		Series 2 Spring rye.		Series 3 Winter rye.
	Water extract.	Diffusion.	Water extract.	Diffusion.	Water extract.
1	0.013	—	0.005	—	—
2	0.018	0.004	—	—	0.010
2½	—	0.013	0.035	0.026	0.045
3	0.096	0.061	0.116	—	0.122
3½	—	—	—	—	0.240
4	0.837	0.372	1.17	0.288	0.768
4½	—	—	—	—	0.877
5	1.42	0.374	1.26	0.209	2.30
5½	—	—	—	—	1.11
6	0.357	0.072	1.87	0.236	0.789
7	0.270	0.003	0.560	0.068	—
8	—	—	0.402	—	—
9	0.234	0.003	—	—	—

Auxin was found in small amounts 2 weeks after anthesis, and then during the next 3 weeks there was a strong accumulation to a maximum at 5 weeks, by which time the original amount at two weeks had increased nearly 80 times. During the sixth week auxin yields fell off sharply, and continued to fall though more gradually.

The results for the spring rye series were similar, though a maximum auxin content was not reached until 6 weeks from anthesis.

Auxin estimations for the vernalised winter rye series were made twice weekly, and the results again demonstrate a very rapid accumulation of auxin reaching a maximum some 5 weeks after anthesis.

The results of the three series are in good agreement, showing appearance of auxin in the grain early in the third week after anthesis, accumulating to a maximum, and then disappearing with ripening. Diffusion experiments gave substantially similar results, the absolute quantities of auxin of course being lower than by extraction and more variable.

1942 experiments.

The main experiment was carried out on autumn-sown winter rye plants grown in the field, with a parallel but similar experiment on spring rye plants grown in pots. The technique employed was to centrifuge the extracts, not filter them, and to estimate for auxin before concentration under reduced pressure. Water extracts and alkaline extracts with a pH 10 phosphate buffer were studied.

The winter rye plants came into flower the first week in June, a fortnight earlier than the corresponding crop of 1941; individual ears were marked with labels on the day of first anther protrusion. The grains were sampled, as far as possible in duplicate, twice weekly throughout their development, for each of the two extraction techniques, water and alkaline hydrolysis. As

the grains enlarged progressively fewer were required for the sample, so that whereas in the first week the grains from two ears were required, by the fifth week 10 or 12 grains sufficed. Samples for the two extraction treatments

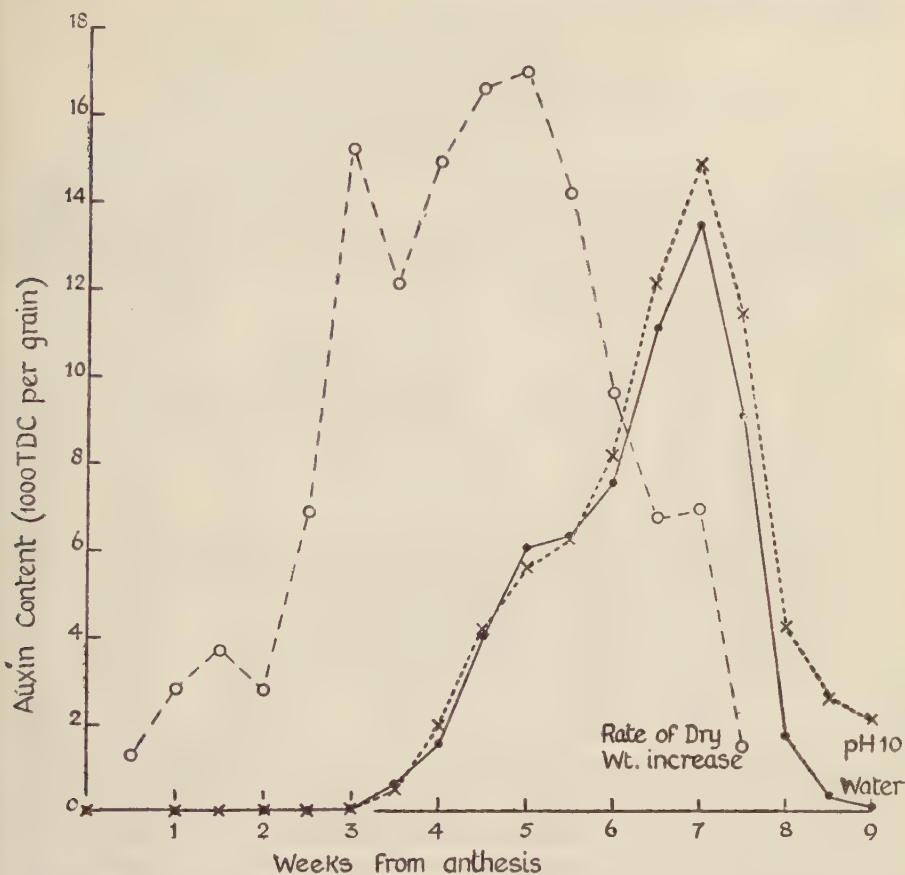


FIG. 1. 1942 experiment. Winter rye. Variation in total auxin content from fertilization to complete maturity of grain in 1,000 T.D.C. units. The data for water extract (black circles) and alkaline extract, pH 10 phosphate buffer (crosses) are given. Open circles show the changes in relative growth rate of the grain.

were strictly paired, using grains from the same ears, but the duplicates came from different ears. The results for this experiment are given in Table VII and graphically represented in Fig. 1.

The marked accumulation of auxin from the third week following anthesis confirmed the 1941 findings, though a higher and a later maximum was reached. The later maximum, 7 weeks as compared with 5 weeks in 1941, was probably associated with the longer development period, for the maximum fresh weight per grain was not attained until over 7 weeks after anthesis, compared with 6 weeks in the previous year.

The considerably higher maximum was likewise due, at least in part, to the

longer developmental period, but this consideration will not completely account for the large difference in auxin content in the two years. In 1941, as already stated, the extract was invariably concentrated first, then diluted

TABLE VII
*Auxin Content of Developing Grain (1,000 T.D.C. units).
1942 Winter Rye Experiment*

Weeks from anthesis.	Fresh wt. per grain	Water (%)	Dry wt. per grain	Auxin Yields per grain						Auxin ratios alkali/water.
	(mg.).		(mg.).	Water			pH 10 phosphate buffer			
	(a)		(b)	Mean.	(a)	(b)	Mean.			
0	0.9	—	0.16	<0.021	6—	<0.021	<0.020	—	<0.020	—
$\frac{1}{2}$	1.7	81.2	0.32	—	—	—	—	—	—	—
1	5.2	—	1.0	<0.019	—	<0.019	<0.020	—	<0.020	—
$1\frac{1}{2}$	10.4	—	2.3	—	—	—	<0.023	—	<0.023	—
2	13.5	—	3.1	<0.015	—	<0.015	0.032	—	0.032	—
$2\frac{1}{2}$	17.4	75.1	4.3	<0.014	—	<0.014	<0.065	<0.010	<0.037	—
3	34.4	—	9.5	0.038	0.070	0.054	0.036	0.043	0.039	0.72
$3\frac{1}{2}$	43.8	69.7	13.3	0.63	—	0.63	0.56	—	0.56	0.89
4	51.6	64.9	18.1	1.39	1.74	1.56	1.78	2.12	1.95	1.26
$4\frac{1}{2}$	64.8	63.3	23.8	4.06	—	4.06	4.20	—	4.20	1.03
5	76.6	63.0	29.5	6.77	5.45	6.11	5.66	—	5.66	0.93
$5\frac{1}{2}$	83.5	57.2	35.7	6.94	5.83	6.38	7.29	5.37	6.33	0.99
6	82.3	52.6	39.0	7.15	8.02	7.58	8.22	8.27	8.24	1.09
$6\frac{1}{2}$	83.3	49.1	42.4	10.09	12.28	11.18	10.09	14.38	12.23	1.09
7	82.7	47.3	43.6	14.62	11.54	13.58	18.63	11.52	15.07	1.11
$7\frac{1}{2}$	85.2	44.4	47.4	9.24	9.08	9.16	9.57	13.57	11.57	1.26
8	69.4	35.8	44.6	1.59	1.93	1.76	4.28	—	4.28	2.46
$8\frac{1}{2}$	58.2	24.3	44.1	0.45	0.32	0.38	2.61	—	2.61	6.87
9	62.5	24.1	47.4	<0.11	<0.11	<0.11	2.12	2.19	2.15	>19.55

to the requisite volume for estimation, whereas in 1942 concentration was resorted to only in the early stages when the original extract was found to be too dilute for successful estimation, and in later samples concentration of the extract was omitted from the procedure. There was evidence to show that concentration of a solution containing auxin with subsequent dilution leads to a loss of auxin, perhaps due to physical change, or a change in solubility, or possibly to adsorption on the glass vessels used. This matter requires further investigation. Avery (1939) and Link and Eggers (1940) have made observations which suggest a similar interpretation.

Comparing extractions with and without alkaline hydrolysis during the period of auxin accumulation it is apparent that there was virtually no pH effect, except perhaps at maximum auxin content when somewhat higher values were reached by the alkali treated. During the ripening period, however, when the auxin rapidly disappeared, differential results were obtained, the final values with alkaline hydrolysis being about 20 times those without such treatment. Disappearance of free auxin (water extractable) is thus almost complete, but there is also very considerable disappearance of total auxin, for the activation by alkaline hydrolysis accounts for only one-seventh

of the auxin present at the peak. Subsequent to this experiment, the optimum alkaline conditions for releasing auxin from the inactive precursor were determined (see above) and it was found that the yield with the pH 10 buffer here employed was more than doubled by using a N/50 NaOH solution at a pH between 12 and 13, giving a recovery of about one-half.

TABLE VIII

Auxin Content of Developing Grain (1,000 T.D.C. units). 1942 Spring Rye

Weeks from anthesis.	Fresh wt. per grain (mg.).	Water (%)	Dry wt. per grain (mg.).	Auxin yield per grain		Auxin ratios alkali/water.
				Water.	Phosphate buffer pH 10	
3	35.6	—	—	0.28	—	—
4	47.9	61.9	18.3	—	2.89	—
4½	50.1	59.6	20.2	—	3.31	—
5	72.2	54.0	33.2	5.81	5.47	0.94
6	69.5	50.6	34.3	9.45	9.10	0.96
7	75.7	45.7	41.1	13.52	—	—

TABLE IX

Auxin Content of Developing Grains (1,000 T.D.C. units). 1943 Winter Rye Experiment

Weeks from an- thesis.	Fresh	Water (%).	Dry	Auxin yields per grain				'Pre- cursor' per grain.	Auxin ratios	
	wt. per grain (mg.).		wt. per grain (mg.).	pH 10 (unbuf- fered solution).	N/50 NaOH	N/10 NaOH	pH 10 water.		N/50 NaOH water.	
0	—	—	—	—	—	—	—	—	—	—
½	3.8	—	—	<0.015	—	—	—	—	—	—
1	6.7	—	—	<0.026	—	<0.026	—	—	—	—
1½	11.3	—	—	<0.014	—	<0.014	—	—	—	—
2	18.2	—	4.2	—	0.097	0.117	—	—	—	—
2½	22.4	—	5.6	0.310	0.325	0.920	—	0.610	—	—
3	25.4	—	7.1	0.130	0.144	0.440	—	0.310	1.11	3.38
3½	39.5	68.1	12.6	0.34	0.33	0.82	—	0.48	—	—
4	59.5	65.5	17.4	—	—	—	—	—	—	—
4½	62.4	64.0	22.5	1.80	1.38	4.12	—	3.32	0.76	2.29
5	63.1	58.0	26.5	2.97	2.77	>6.80	—	>3.83	0.94	2.29
5½	—	—	—	—	—	—	—	—	—	—
6	75.4	51.3	36.7	3.01	3.80	12.00	—	8.99	1.26	3.99
6½	79.6	53.8	36.8	5.55	7.00	13.08	—	7.53	1.26	2.36
7	83.0	46.1	44.7	6.35	8.10	17.10	18.67	10.75	1.28	2.69
7½	68.1	42.2	39.4	4.66	4.80	19.08	14.70	14.42	1.03	4.09
8	68.3	37.3	43.7	0.83	1.08	5.40	7.70	4.57	1.30	6.51
8½	63.3	21.5	49.8	0.10	0.51	7.98	6.60	7.88	5.10	79.80

In the subsidiary experiment with spring rye, it was found that the course of auxin production was essentially the same, qualitatively and quantitatively, as with winter rye, as is seen by comparing Tables VII and VIII.

1943 experiments.

The object of the main experiment of 1943 was to study the effect on auxin yield of alkaline hydrolysis when carried out under optimal conditions. The extraction methods used were (i) water extraction, (ii) alkaline hydrolysis with unbuffered pH 10 NaOH solution, (iii) alkaline hydrolysis with N/50 NaOH solution, and (iv) alkaline hydrolysis with N/10 NaOH solution.

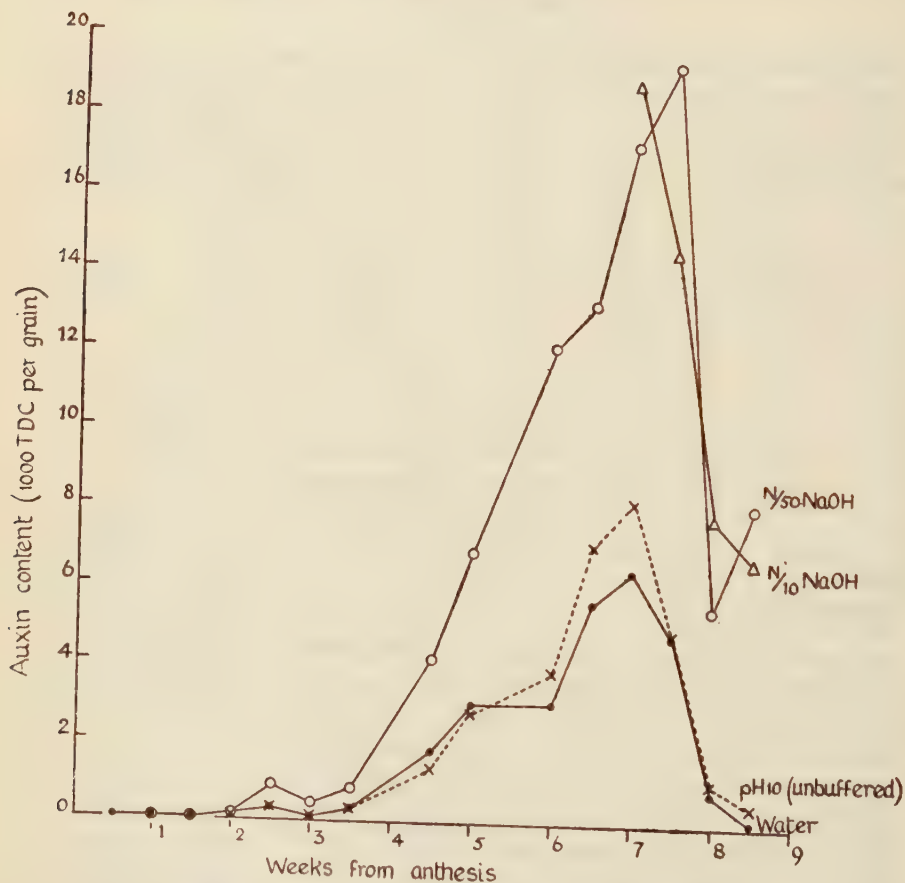


Fig. 2. 1943 experiment. Winter rye. Auxin content during the development of the grain from fertilization to complete maturity. The data are given for four methods of extraction, water, NaOH solution pH 10, N/50 NaOH solution, and N/10 NaOH solution. A peak value occurs in all cases at 7 to 7½ weeks.

Autumn-sown winter rye was again used, flowering occurring very early (May 26–28), and sufficient ears were labelled on May 28 to suffice for the whole experiment. Samples were taken at half-week intervals throughout development, and the results are presented in Table IX and Fig. 2.

The general result confirms earlier experiments. Auxin appeared some 2 weeks after anthesis, accumulated to a maximum—this time at 7½ weeks—

and disappeared rapidly in the final ripening stage. Comparing water extraction with pH 10 alkaline extraction there was no obvious difference in auxin yield previous to the final ripening stage except for the tendency for alkaline values to be higher at the auxin peak. With ripening the pH effect appeared, though not to the same extent as in 1942, due to the fact that a buffered solution is more effective in releasing auxin than one unbuffered (see Table III). The yields from the N/50 NaOH extracts were consistently higher than those from either water or pH 10 solution throughout development of the grain, the ratio of the N/50 NaOH and water yields being of the order of 3 until ripening set in, when with the almost complete disappearance of the free auxin, accompanied by partial disappearance of the total auxin, this ratio rose rapidly to the order of 80.

The three years' experiments have shown quite conclusively that in the rye grain as it develops free auxin begins to form in detectable amounts early in the third week, accumulates at a very high rate to a maximum, and disappears almost entirely during the final ripening stages. As well as free auxin there is an inactive substance ('precursor') present from which auxin is released under alkaline conditions. The variation in 'precursor' content per grain is shown in Table IX and represents the differences in free auxin (water extract) and total auxin (extract with N/5 NaOH). These figures show that 'precursor' content reaches a maximum at the same time as the free auxin and then less rapidly falls to a value approximately half that at the peak. Up to the peak of auxin content the relative concentration of 'precursor' and free auxin remains nearly constant, as is seen in the column of auxin ratios in Table IX. In the phase of falling auxin content the loss in free auxin is much greater than that in precursor, so that the ratio of extract by alkali and water rises rapidly to a very high value; these relations are shown in Table IX.

Certain other facts concerning the production of auxin in the grain emerge when comparisons are made with fresh and dry weight data, and water contents during development. In the first place there is no direct proportionality between grain weight and auxin content. By the time that auxin can be detected during the third week, the grain itself has increased in dry weight from under one-fifth of a milligram to over 5 mg., and subsequently dry matter increases only tenfold, whereas auxin increases more than fifty-fold. It should, however, be noted that the auxin peak corresponds with the attainment of maximum dry weight. The relation between change in rate of dry-weight increase as compared with auxin content is shown in Fig. 1. It is seen that maximum rate of dry-weight increase occurs before much accumulation of auxin, whereas at the peak value of auxin the rate of dry-weight increase has fallen practically to zero, indicating that the grain has already attained its final dry weight.

The relative water content of the grain falls throughout development, from over 80 per cent. at fertilization, to about 70 per cent. when auxin first appears, about 50 per cent. at the auxin peak, about 40 per cent. when the ear is ready for harvest, and finally to about 12 per cent. in the fully ripe

grain. During ripening, when auxin is disappearing rapidly, there appears to be an acceleration in the drying of the grain, and the consistency of the endosperm tissue changes noticeably from a soft milky stage to a much firmer dough stage. There may be no direct relation between these physical changes on the one hand, and the disappearance of auxin on the other, but indirectly they must be connected. The pericarp outer tissues of the grain also undergo marked changes, and the full, smooth, greenish-yellow grain gives place to the thinner, rough, yellowish-brown grain. At this stage the grain attains the power to germinate, and this may afford a clue to the relationship between auxin disappearance, accelerated drying-out, and ripening.

In a series of germination tests with grains at various stages of development, it was found that grains before the auxin peak would not germinate, grains at the auxin peak sometimes germinated though slowly and sporadically, while grains a week beyond the peak all germinated, though still slowly compared with fully ripe grains. It was also observed that grains just prior to the auxin peak could be stimulated to germinate by making punctures with a needle in the pericarp near the embryo, suggesting that permeability of the pericarp tissues to gaseous exchange is requisite for germination, a condition normally attained in ripening by the drying-out of the grain. The factors determining whether or not an unripe grain will germinate (cf. Bishop, 1944) would thus appear not to be inherent in the embryo itself; indeed this has been conclusively demonstrated. From 4-week grains, well before the auxin peak, and thus as a whole incapable of immediate germination, the embryos were excised and cultured on nutrient agar; some of these embryos developed without any delay into normal plants, proving that the power of the developing grain to germinate is dependent, not upon the embryo, but on the pericarp tissues, which, until they become permeable to gaseous exchange, are fully effective in inhibiting germination. Summarizing these findings it appears that: (1) the germination of the embryo is not dependent upon the auxin content of the grain but upon an ample oxygen supply; (2) disappearance of the total auxin is related to the stage in development at which the pericarp becomes permeable to gases. Whether there is a causal relationship between these factors, whether oxygen access or water loss is so related, is not at present known, but investigations to elucidate these points are in progress.

What does appear to be the case is that the disappearance of auxin is associated with ripening, and factors which delay ripening also delay auxin disappearance. In 1941 the autumn-sown winter rye plants ripened quickly and were harvested 6 weeks after anthesis, the auxin peak having been reached a week earlier. In 1942 and 1943 plants were not ready for harvest until the eighth week after anthesis, and the auxin peaks were correspondingly later. The further observation has been made that grains removed from the ear prior to attaining their auxin peak, and kept in Petri dishes, ripen off more slowly, and manifest a delay in the disappearance of their auxin when compared with grains left in the harvested ear. That these grains nevertheless lose their auxin when severed from the ear demonstrates that the auxin does

not migrate from the grain but disappears *in situ*. This leads to the conclusion that the final disappearance of the auxin is one of several changes occurring in the grain which can be associated with the general process of ripening.

AUXIN CONTENT OF PREMATURELY HARVESTED GRAIN

The experiments now to be described investigate the effect of premature harvest of auxin formation in the grain. The relation to the general development of the plant has been studied by Nutman (1939, 1941), who followed up an observation of Gregory and Purvis (1938) that rye ears harvested as early as 5 days after anthesis and dried down at ordinary temperatures, produced dwarf grains which were viable. Nutman found that in ears harvested at progressively later stages the ripe grains exhibited a gradual transition from a dwarf condition to that of the normal grain, the size of the ripe grain depending directly on the time of harvest, there being virtually no change in dry weight subsequent to harvest. Within the grain, however, developmental processes continued, notably the growth and development of the embryo, while outwardly the grain exhibited the typical signs of ripening. The general effect of premature harvest is thus to bring to a standstill the processes concerned with increment in dry matter, without interfering with the normal sequence of developmental changes, except that these are accelerated. An examination of the auxin relations in such prematurely harvested grain was therefore undertaken, and the results obtained have shown that auxin formation is essentially a concomitant of development, and is independent of dry matter accumulation.

The results of the 1941 experiments are summarized in Table X. Ears were harvested at 2 and 4 weeks after anthesis, thus before and during auxin accumulation respectively. In the case of the 4-week harvest grains were also

TABLE X

*The Effect of Premature Harvest on Auxin Content of Rye Grains.
1941 Experiments. Auxin unit 1,000 T.D.C.*

Time of harvest from anthesis . . .	2 weeks.				4 weeks.	
	Winter.		Spring.		Winter.	
Variety . . .	Extraction.	Diffusion.	Extraction.	Diffusion.	Extraction.	Diffusion.
Method . . .						
At harvest . . .	0.018	0.008	0.020	—	0.865	0.372
1 week after harvest in ear	0.109	0.014	0.080	—	0.080	0.024
1 week after harvest in Petri dishes . . .	—	—	—	—	1.087	0.322
2 weeks after harvest in Petri dishes . . .	—	—	—	—	0.100	0.024
Left on plant for a further week after harvest sample removed . . .	0.096	0.061	0.116	—	1.419	0.374

dissected from the ear and kept in Petri dishes in an incubator at 20° C. Auxin was estimated by diffusion and water extraction.

In grains of intact ears harvested 2 weeks after anthesis, at a stage when auxin may just be detected by water extraction, auxin increased in quantity during a week at approximately the same rate as in control unharvested ears. This was the case both for winter and spring rye. Diffusion experiments also showed an auxin increase though the observed amount was less than in the controls.

In grains of intact ears harvested 4 weeks after anthesis, at a stage when auxin is accumulating, auxin decreased in one week to a fraction of the original amount. This contrasted with grains dissected from the ear and kept in a Petri dish, for in these auxin increased in amount, though not to the same extent as in unharvested ears. A second extraction of the dissected grains after a further week showed this initial rise in auxin content to be only a temporary effect, for during the second week the auxin largely disappeared. Diffusion tests gave a similar result. This delayed disappearance in the dissected grains is associated with a less rapid ripening-off of the grains, as was mentioned above (see page 250).

TABLE XI

Effect of Premature Harvest on the Auxin Content of Rye Grains
(1,000 T.D.C. units)

1942 Experiment. H = Prematurely-harvested grain
C = Control, unharvested grain

Weeks from anthesis.	Auxin yields									
	Dry wt.		Water content (%)		Phosphate buffer pH 10				Auxin ratios alkali/water	
					Water					
					H.	C.	H.	C.		
3	9.53	9.53	72.3	72.3	0.054	0.054	0.039	0.039	0.72	0.72
3+1	7.60	18.1	65.9	64.9	0.64	1.56	0.85	1.95	1.33	1.24
3+2	8.83	29.5	50.1	62.0	0.77	6.11	1.06	5.66	1.37	0.93
3+3	9.58	34.0	22.1	52.6	0.13	7.58	1.37	8.24	10.54	1.09

In 1942 a collection of winter rye ears was made 3 weeks after anthesis, soon after auxin had appeared in the grains. Estimations with and without alkaline hydrolysis (pH 10 buffer) were made immediately, and then at weekly intervals until the prematurely harvested grains were ripe. The results in Table XI show that auxin continued to accumulate after harvest, but at a lower rate than in the unharvested controls. For 2 weeks after harvesting, the ears with water extraction and alkaline hydrolysis gave values of the same order, but during the third week free auxin largely disappeared, and the pH effect was apparent. In the controls at this time, 6 weeks after anthesis, auxin was still accumulating, and water extraction was giving yields similar to alkaline extraction. The water content of the prematurely harvested grain during

this third week fell from 50 to 22 per cent. as compared with from 62 to 53 per cent. in the controls. It may be said, therefore, that harvest 3 weeks after anthesis is followed by reduced auxin accumulation and accelerated ripening.

TABLE XII
Effect of Premature Harvest on Auxin Content of Rye Grain.
(1,000 T.D.C. units)

1943 Experiment. H = Prematurely harvested grain
C = Control, unharvested grain

Weeks from anthesis.	Dry wt.		Water content (%)		Auxin yields				Auxin ratios alkali/water	
					Water		N/50 NaOH			
	H.	C.	H.	C.	H.	C.	H.	C.		
2	4.2	4.2	77.0	77.0	—	—	0.117	—	—	—
2+1	3.9	7.1	70.8	72.0	0.106	0.130	0.442	0.440	4.17	3.38
2+2	2.6	17.4	65.5	65.5	0.180	1.10	0.354	2.50	1.97	2.27
2+3	2.4	26.5	35.7	58.0	0.020	2.97	0.457	6.80	22.85	2.29
3	7.1	7.1	72.0	72.0	0.130	0.130	0.440	0.440	3.38	3.38
3+1	10.1	17.4	64.4	65.5	0.45	1.10	0.49	2.50	1.09	2.27
3+2	11.2	26.5	46.7	58.0	0.70	2.97	2.04	6.80	2.91	2.29
3+3	9.3	36.7	27.3	51.3	0.027	3.01	1.12	12.00	41.48	3.99
4	17.4	17.4	65.5	65.5	1.10	1.10	2.50	2.50	2.27	2.27
4+1	16.4	26.5	54.2	58.0	1.48	2.97	2.77	6.80	1.87	2.29
4+2	17.5	36.7	48.2	51.3	0.223	3.01	1.52	12.00	6.82	3.99
4+3	17.2	44.7	17.4	46.1	0.068	6.35	1.98	17.10	53.51	2.69
5	26.5	26.5	58.0	58.0	2.97	2.97	6.80	6.80	2.29	2.29
5+1	22.7	36.7	48.2	51.3	1.01	3.01	3.08	12.00	3.05	3.99
5+2	20.7	44.7	28.3	46.1	0.068	6.35	1.37	17.10	20.15	2.69
7½	39.4	39.4	42.2	42.2	4.66	4.66	19.08	19.08	4.09	4.09
7½+1	49.8	—	21.5	—	0.10	—	7.98	—	79.80	—

In 1943 a comprehensive experiment was undertaken with winter rye. Ears were harvested at 2, 3, 4, and 5 weeks after anthesis, and auxin contents of grain, using water and N/50 NaOH for extraction, were determined weekly until ripening of the harvested ears occurred. The results are summarized in Table XII, and total auxin contents (auxin plus 'precursor') are plotted in Fig. 3. For each harvest notes are now appended, stating auxin content, relative quantities of alkali and water-extractable auxin, and water content.

2-week harvest. During the first week after harvest, free auxin formed at the same rate as in controls, but thereafter more slowly, and during the third week disappeared; the ratio of total auxin to free auxin increased sharply to over 20 during the third week; water content fell at the same rate as controls until the third week, and then more rapidly.

3-week harvest. Auxin increased at a much lower rate than in controls left on the plant, disappearing in the third week after harvest, when the ratio total auxin : free auxin increased to over 40; water content fell off more rapidly than controls in the second week, but was not very different until the third week.

4-week harvest. Auxin increased to a small extent during the first week after

harvest, and then disappeared, the ratio total auxin : free auxin started to rise in the second week, and reached over 50 during the third; water content was less than in unharvested controls from the first week.

5-week harvest. Auxin decreased during the first week from harvest and was below the limits of detection after 2 weeks, at which time the ratio

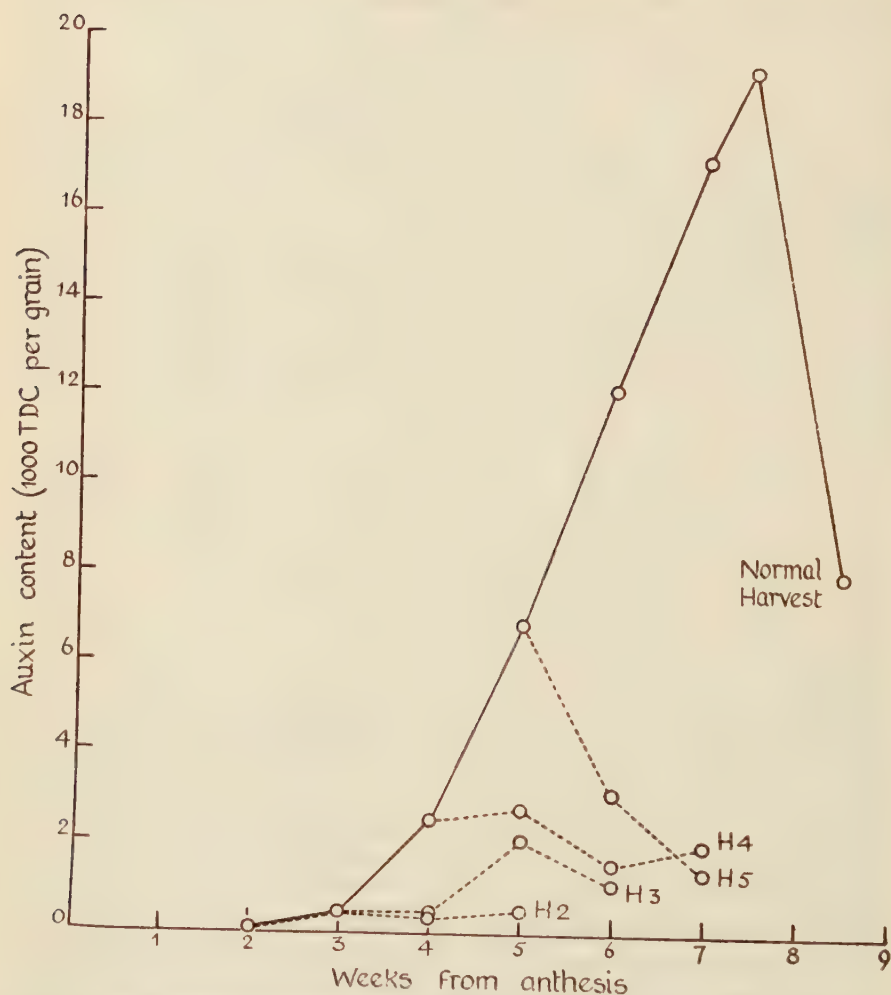


FIG. 3. The effect of time of harvesting on accumulation of total auxin (N/50 NaOH extraction). Results for 2, 3, 4, 5, and $7\frac{1}{2}$ weeks (normal harvest) are shown. In each case after reaching a peak value the auxin disappears. The level attained rises with delay in harvest.

total auxin : free auxin exceeded 20; water content was less than controls from the first week.

$7\frac{1}{2}$ -week harvest. This was the normal harvest, after which auxin content decreased rapidly, the ratio total auxin : free auxin increased to 80, and water content fell to 21 per cent. in one week.

Comparing the various harvests the following relations are seen: (1) In the prematurely harvested grain the auxin cycle is of the same kind as occurs during the maturation of the normal grain on the plant; a phase of rising auxin content attaining a peak value, followed by a disappearance of auxin as the grain loses water and ripens. The loss is greatest in free auxin, but the auxin no longer extractable by water is only partially recovered by alkaline hydrolysis, so that the total auxin also diminishes with ripening of the grain. The relatively more rapid loss in free auxin is seen in the trend of the ratio alkali : water-extractable auxin in column 10 of Table XII. (2) The earlier the harvest the less marked is the phase of accumulation of auxin, and the

TABLE XIII

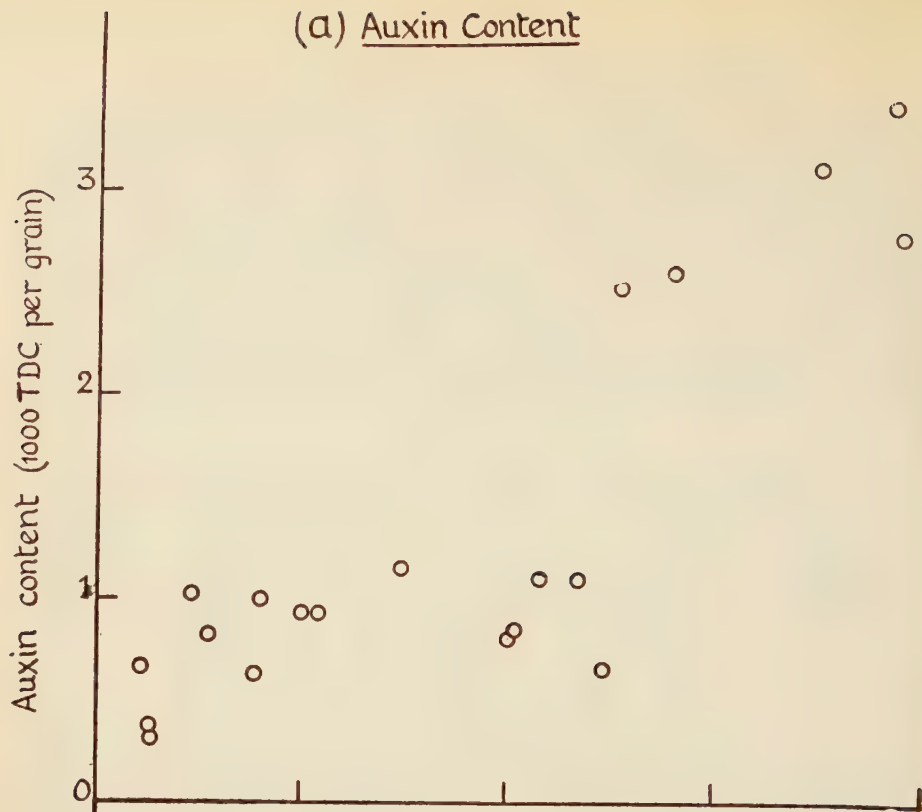
Protein Content of Ripe Grain of Winter Rye, prematurely harvested

Time of harvest (days after anthesis).	Wt. per grain (mg.).	Water (%).	Dry wt. per grain.	Percentage protein			Wt. of protein per grain (mg.).
				(a)	(b)	Mean.	
12	3.83	9.3	3.47	19.3	20.3	19.80	0.69
20	10.08	7.2	9.28	13.3	13.1	13.20	1.22
26	18.68	9.6	16.89	9.7	9.4	9.53	1.61
35	26.51	8.8	24.17	10.3	10.2	10.25	2.47
43	38.83	8.8	35.43	6.9	6.8	6.85	2.43

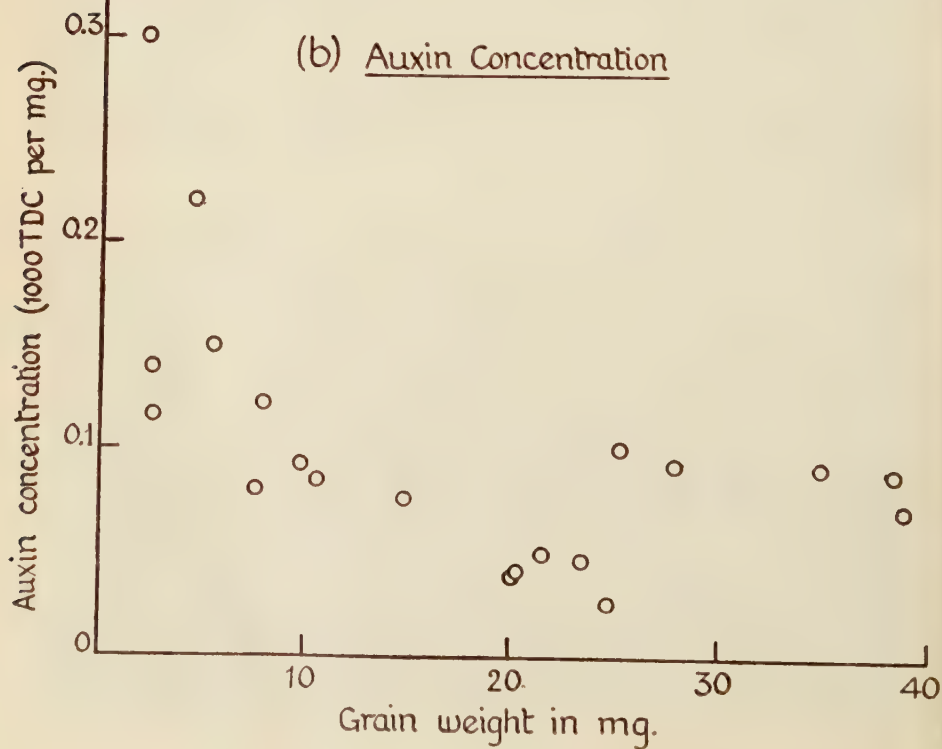
lower the peak value. (3) In the harvests later than the 4 weeks after anthesis loss of auxin is in progress within a week of harvest, being accompanied by a rapid water loss. (4) The loss of total auxin in ripening is relatively less in the earlier harvested ears, which results in the most dwarfed grain having the highest concentration of auxin, as was established in a series of determinations on fully ripe grain of various sizes obtained by serial removal of ears. These estimations involved hydrolysis with an alkaline solution buffered at pH 10, and did not give maximum yields of auxin. The results are presented graphically in Fig. 4, *a* and *b*.

In Fig. 4*a* auxin contents, and in 4*b* auxin concentrations, are plotted against grain weight. Auxin content varies relatively little for the ripe grain from 2 to 20 mg. weight, but then appears to increase abruptly, due to the very strong accumulation of auxin during the middle period of development prior to ripening. Auxin concentration (units per mg.) decreases from a maximum in the smallest grain to a minimum in grains from 20 to 25 mg., and then possibly rises again in the largest grains. The underlying cause is no doubt the very different effects of premature harvest on dry weight and development; the former is abruptly checked, the latter is essentially unaffected. A further result of premature harvest is seen in its influence on the relative amount of protein. The most dwarf viable grain approximates in length to the normal grain, but is crinkled and shrunken owing to the small amount of stored carbohydrate. The aleurone layer, however, is well developed, and the percentage protein in a series of ripe prematurely harvested grain was found to fall from about 20 per cent. in grains of 4 mg. to 7 per cent. in grains of 40 mg. as seen in Table XIII. The fact that auxin is much more

(a) Auxin Content



(b) Auxin Concentration



concentrated in the aleurone layer than in the endosperm proper (see above) suggests a relationship between protein and auxin.

AUXIN CONTENT OF THE EAR DURING DEVELOPMENT

In addition to the individual grain the ear as a whole was examined, primarily to discover if migration from one organ to another played any part in auxin relations of the grain. To this end in the main 1942 experiment, already described, sampling for auxin estimation began at a stage prior to ear emergence. The results are summarized in Table XIV.

TABLE XIV

Auxin Content of the Ear during Development. 1942 Experiment
(1,000 T.D.C. units)

Region of ear.	Stage of development.	Auxin yields		Auxin ratios alkali/ water.
		Water.	Phosphate buffer, pH 10.	
Whole ear	Before emergence	< 2.10	< 2.06	—
—	—	—	< 1.80	—
—	—	—	< 0.102	—
—	Early emergence	—	< 1.54	—
—	Anthesis	—	22.05	—
<i>Ear Analysis</i>	Pre-anthesis			
Rachis	—	—	< 1.60	—
Glumes	—	< 1.34	< 2.36	—
Ovaries and stigmas	—	< 1.36	< 1.36	—
Anthers and pollen	—	62.56	71.06	1.14
Single ovary	—	< 0.020	< 0.021	—
Single anther	—	0.307	0.359	1.14
Anthers	Green and unripe	—	1.64	—
—	Yellowing and ripe	—	0.066	—
—	Dehiscent	—	< 0.022	—
—	Ear emerging; dark green	—	0.116	—
—	Ear emerged; green	1.13	1.43	1.27
—	Near anthesis; yellowing	0.257	0.224	0.87
—	Pollen (per anther)	—	< 0.020	—

Extraction of whole ears at three stages, pre-emergence, early emergence, and anthesis, showed that auxin had appeared in the ear at some time between emergence and anthesis. To locate this auxin, ears at a stage immediately prior to anthesis were used, and rachis, glumes, carpels, and anthers were separately extracted. It was thus found that the site of auxin production in the ear at this early stage was in the anthers.

A study was next made of the anthers, and extractions were made at three stages, namely green and unripe, yellowing and ripe, and after dehiscence and without pollen. The unripe anther was found to contain considerable amounts

FIG. 4. Regression of (a) auxin content, (b) auxin concentration, on grain weight, for ripe grain harvested at various times after anthesis.

of auxin, the ripe anthers much less, and after dehiscence no auxin was detected. Further extractions at various stages between emergence and anthesis showed that auxin appears in the anthers just as the ear emerges from the flag leaf sheath, increases to a maximum, and disappears with ripening of the pollen. These findings bear a resemblance to the cycle later occurring in the developing carpel. At the stage at which auxin appears in the anther the pollen grains are colourless and have thin walls. During ripening the yellow colour develops with the formation of a thick and waxy extine. Ripe pollen itself failed to yield auxin when extracted with water or alkali, and in this respect differs from the ripe grain in which part at least of the auxin lost during ripening is recoverable by alkaline hydrolysis. It should be noted too that the very rapid elongation of the stamen filaments constituting anthesis does not coincide with the auxin peak in the anthers.

In 1943 more estimations were made applying the optimum conditions for alkaline hydrolysis. Essentially the same results were obtained, highest auxin yields in the unripe anthers and no detectable auxin in the pollen grains. Even N/50 NaOH failed to extract auxin from pollen, though with unripe anthers higher yields were obtained, the ratio alkaline-extractable auxin: water-extractable auxin, being about 3.

In the ear there are thus two sites of auxin formation, anthers and carpels, with certain features in common, namely the accumulation of auxin relatively late in development, and subsequent disappearance with ripening. The fact that auxin was not detected in other regions of the ear, and that auxin formation in the anthers occurred 2 or 3 weeks earlier than in the carpel, strongly suggests that two distinct auxin systems are involved. This confirms the general finding that the auxin of the developing grain is produced *in situ*.

DISCUSSION

Nature of auxin production in the developing grain.

The foregoing experiments have shown that the free auxin content of the mature rye grain results from two processes, active accumulation during development, followed by almost complete disappearance during ripening. An inactive 'precursor' substance from which auxin is released by alkaline hydrolysis is also present and its production runs parallel with that of the auxin, so that throughout the period of accumulation some twice as much of the precursor is present (Table IX). This might be accounted for either (a) by an equilibrium between precursor and free auxin throughout development, or (b) independent production of the two substances but with different velocity constants. The second alternative would presuppose a common origin of the two substances, in other words a further 'precursor'.

During ripening, after the auxin maximum is passed, both free and precursor auxin disappear, but at different rates, so that the ratio of 'precursor' to free auxin rapidly rises (Table IX). There is thus in this phase no evidence for an equilibrium between the two fractions. There may possibly be a

reconversion of free auxin to precursor, though were this so one might expect the amount of precursor to increase at the expense of the free auxin. In fact the precursor itself decreases during ripening to little more than half the amount extracted at its peak concentration.

The disappearance of the auxin may interrupt the phase of accumulation, for if ears are harvested prematurely the grains ripen off more quickly, displaying attendant changes in the auxin-precursor balance. For a time after premature harvest, if this is carried out early enough, auxin accumulation proceeds, though on a reduced scale, and this dissociates auxin production from the processes of dry-weight accumulation, and links it with the developmental processes which occur even after the ear has been harvested. From this it is inferred that the auxin relations of the grain are an intimate part of its development, and just as the sequence of developmental changes is maintained in the prematurely harvested grain with the formation of dwarf embryos (Nutman, 1941), so is the sequence of auxin accumulation and disappearance.

It is pertinent at this point to co-ordinate these auxin phenomena with the other developmental processes already studied by Nutman (1939, 1941). It is to be remembered that auxin first appears in the grain in detectable amount early in the third week from anthesis, accumulates rapidly during the next 3 or 4 weeks to a peak value, and then disappears. The maximum rate of growth of the grain coincides with the point in time at which measurable quantities of auxin and precursor first appear in the carpel; while the peak of auxin accumulation occurs at the time when growth of the fruit has ceased. These relations are shown in Fig. 1. It is clear that the growth rate bears no direct relation with the concentration of either auxin or precursor in the tissues.

The growth of the embryo-sac was shown by Nutman to be discontinuous with a period of very slow growth at 8 to 10 days from anthesis separating two periods of rapid growth. By the beginning of the third week the rate of increase in length is already falling off, and only then does auxin begin to accumulate. The difference observed by Nutman between spring and winter rye is limited to early development, the two types attaining the same final size, which is in accordance with the fact that no significant difference has been noticed in their auxin contents.

In a like manner the embryo has completed its phase of exponential growth before the production of large quantities of auxin, so it is a characteristic of auxin accumulation that it occurs relatively late in development after the more important changes in various regions of the carpel are complete.

Nutman postulated the formation of growth-promoting substances in the developing grain which he associated with the degeneration of certain tissues at the expense of others. There was no evidence, however, that these growth substances were of the auxin type. One observation by Nutman requires stressing. During the dissolution of the endosperm in the proximity of the scutellum a layer of depleted cells was noted similar to that described in barley by Brown and Morris (1890). The data in Tables IV and V show that in this region the highest concentration of auxin in rye grain is to be found.

The results with rye may be compared with data obtained from other plants, though few other studies of auxin production during seed development have been made. Using alcohol as the extracting solvent Laibach and Meyer (1935) studied maize and sunflower throughout their ontogeny. In the fruit of both they observed an accumulation of auxin followed by disappearance with ripening, resembling the relations seen in rye. Maize has also been studied by Avery et al. (1942) and Wittwer (1943). The latter used the same methods as Laibach and Meyer, obtaining the same result, but Avery et al., using water extraction and alkaline hydrolysis, supplemented this information with data for a precursor.

Several important differences exist between maize and rye. In maize there is always an overwhelming predominance of precursor over free auxin with no obvious shift in their relative proportions even to the completely ripe stage; some 5 to 10 per cent. of the total is free auxin. In rye there is relatively much more free auxin present during the phase of accumulation (from 30 to 40 per cent. of the total), but during ripening this value falls to approximately 2 per cent. representing a sharp shift in the relative proportions of free auxin and precursor. A second contrast between the two cereals is in the loss of total auxin during ripening. In maize the loss is about four-fifths of the total, in rye only about a half. A third difference is in the effect of premature harvest. In maize, to judge from Avery's diagram, the disappearance of total auxin in normal ripening on the plant is shared by the free auxin and precursor fractions, so that their relative concentrations remain unchanged; indeed if there is any difference a greater relative reduction may occur in the precursor. Following premature harvest at the auxin peak, however, and drying down the grain at air temperature, Avery found that the total loss is reduced from 80 to 25 per cent., though this reduction is limited to the precursor, with the free auxin disappearing to its former extent. The consequent increase in the ratio of precursor to free auxin resembles the change in their proportions characteristic of rye in normal or premature harvest. In maize, therefore, the relation of precursor to free auxin remains constant if the grains ripen on the plant, with premature harvest leading to an absolute and relatively less loss in precursor. In rye ripening always leads to a predominant loss of free auxin whether harvest is normal or premature.

Other auxin studies of developing seeds, exemplified by the work of Gustafson (1939 and 1939 *a*), bear on the problem of parthenocarpy and need not be discussed.

Auxin systems of the grain.

In general two auxin systems have been recognized in plant tissues, one which includes the auxins *a* and *b*, the other being the indole-3-acetic acid system. These substances can be distinguished by the different stability of their solutions, for auxin *b* is unstable except in neutral solution, auxin *a* is stable in acid solution, and indole-acetic acid in alkaline solution. Haagen-Smit et al. (1942) have isolated indole-acetic acid from the maize grain, and

this has been confirmed by Berger and Avery (1944). In addition a precursor substance has been demonstrated by the release of auxin in alkaline hydrolysis (Avery et al., 1941; Haagen-Smit et al., 1942; Thimann et al. 1942). Recently this precursor has been extracted from maize endosperm, and following alkaline hydrolysis indole-acetic acid has been obtained (Berger and Avery, 1944, 1944 *a*). Further it has been shown by Avery et al. (1941, 1942, 1942 *a*) and the present author that under alkaline conditions auxin is released from the grains of all the common cereals, and there is no doubt of the presence in the cereal grain of the indole-acetic acid system of hormone. It appears, however, from the early extraction of auxin *a* from maize germ oil, using ether as the original solvent (Kögl et al., 1934), that the second system is also present in the grain.

In general also plant tissues fall into two classes with regard to extractability of growth substances. With the exception of developing anthers and carpels, and mature seeds, organic solvents such as ether have been found to extract growth hormone, whereas water as a solvent has failed. As seen in Table I, ether also extracts growth hormone in varying degrees from the grains of the cereals studied, but in all cases is inferior to water. These facts cannot be attributed to the insolubility of auxin *a* in water, for though this hormone has come to be regarded as typical of developing tissues, the demonstration of its presence in the coleoptile by Went (1928) depended upon diffusion into an aqueous medium. Probably the success of ether in extracting hormone from such tissues may be due to the association of the hormone with a fatty phase inaccessible to water. The data in Table I for maize shows considerable ether solubility, which might be due to the fact that the auxin *a* complex is present and can be extracted in a way similar to that for developing tissues. In the rye grain, on the other hand, proportionately much less ether extraction occurs, so that on the assumption made most of the growth-promoting substance present is not auxin *a* but indole-acetic acid. As noted above, however, the presence of auxin *a* in the cereal fruit has been proved by the isolation of this hormone from the germ oil of maize (Kögl et al., 1934). Similarly Thimann (1934) succeeded in extracting an active substance from the germ oil of wheat, using chloroform as solvent. From these facts the presence of auxin *a* in the cereal embryo is to be inferred. In the present work the water-extractable hormone from the rye embryo was too small in amount to estimate, so that the indole-acetic acid must occur in very low concentration if at all; while in maize it has been shown to be of the order of 0.2 per cent. of the total hormone of the grain. The presence of two hormone systems in the grain is therefore not excluded, the auxin *a* system predominating in the embryo, and the indole-acetic acid system in the aleurone and endosperm; but in any event the indole-acetic acid is present in far higher concentration. It appears probable too that the hormone concerned during development of the carpel and anther is of the indole-acetic acid type.

The presence of a precursor extractable in water and yielding indole-acetic

acid on alkaline hydrolysis has been demonstrated in the developing carpel from the third week after fertilization. The relation of free auxin to precursor has already been discussed, and in the anther as in the carpel an approximate ratio of 1 to 2 auxin to precursor obtains during the period of accumulation up to the peak value. The subsequent relations differ in the two regions. In the ripe carpel free auxin can be recovered by alkaline hydrolysis, but as the pollen ripens the precursor disappears and is no longer convertible to free hormone by conditions of alkalinity. Whether this is due to failure to extract it from the ripe pollen remains uncertain.

Nature of the precursor.

The position with regard to hormone precursors has been very considerably clarified by the recent work of Berger and Avery (1944 and 1944 *a*), who have isolated a substance by extraction with 50 per cent. acetone-water solution, and have shown that on alkaline hydrolysis indole-acetic acid is produced. Whether in fact this substance functions in the plant as source of growth-promoting substance cannot at this stage be finally decided. It is remarkable, however, that no enzymatic release of indole-acetic acid has yet been achieved. Berger and Avery's suggestion that the precursor is intimately associated with protein, possibly by adsorption, would go far to explain some of the facts recorded in this paper. For with varying quantities either of the specific protein or of total indole-acetic acid produced in the tissues, the ratio of free to adsorbed hormone would remain constant, as occurs to the peak value in both anther and carpel of rye. The same relation holds in maize to maturity, although here relatively much more auxin is adsorbed. In this connexion the high proportion of hormone found in the aleurone layer of rye (71 per cent. of the total, see Table V), in proximity to the main protein reserves, is suggestive. In rye, however, as water content of the grain falls during ripening, the ratio of free hormone to precursor falls to a low value. This is also in accordance with the adsorption hypothesis, for with falling water content concentration of free hormone rises and therefore a relatively larger proportion is adsorbed. The disappearance of total hormone in ripening, on the other hand, cannot be accounted for by the same hypothesis. In view of the facts of the case the term 'precursor' is misleading since there is no evidence to date that the 'precursor' is formed before free hormone appears, which is what the term implies. Indeed even in the germinating grain it still remains to be shown that the stored 'precursor' is translocated to the coleoptile and that the reserves in the grain disappear during germination. It should also be noted that the purest form of precursor prepared by Berger and Avery has failed to display growth-promoting activities in every test used.

The function of the auxins in development.

The hypothesis put forward by Wittwer (1943) and Murneek and Wittwer (1943) associates auxin production in the anther with reduction division, and in the carpel with fertilization. The growth-promoting substances so set free

are believed to promote vegetative growth of the plant and evidence to substantiate this is put forward by Wittwer (1943). In rye, however, this suggestion scarcely meets the case. The vegetative growth in cereals is of two types, the rapid production of laterals during tillering and the rapid extension of the stem during 'shooting' prior to ear emergence. It is well known that tillering ceases at the time when the ear is differentiated at the stem apices before reduction division has occurred either in the anther or carpel. Maximum extension growth is also complete before fertilization occurs. The data in this paper show conclusively that no detectable auxin is present in the ear before emergence, at which time 'shooting' is nearing completion; indeed in the carpel auxin accumulation is deferred for a further three weeks. The data of relative growth rate from Briggs, Kidd, and West (1920 and 1920 *a*) are somewhat freely interpreted by Wittwer (1943, Fig. 10), and the transient increase attributed to synapsis and syngamy may well be due to the grand period of growth of the ear with the attendant increase in dry weight due to the high assimilation rate of this organ (Archbold, 1942).

With regard to vernalisation, the mechanism of which this work set out to elucidate, it can be categorically stated that the hormones investigated bear no relation to the problem. First, no significant difference has been established between the auxin contents, at maturity and throughout development, of spring and winter rye grains. Second, no auxin has been detected in the rye embryo either throughout development in the ear or during germination whether at normal or vernalisation temperature. This is directly contrary to the assumption of Cholodny that auxin accumulates in the embryo during vernalisation, to which accumulation he attributed the after effects of low temperature.

SUMMARY

The auxin relations of the developing ear and grain of rye have been investigated using the standard *Avena coleoptile* technique. After preliminary experiments water was adopted as the extracting solvent for the main developmental work.

Mature cereal grains vary considerably both in absolute auxin content and in auxin concentration. Maize is richest in auxin with oat, rye, wheat, and barley forming a descending series (Table I). As a solvent water is preferable to ether, chloroform, and alcohol, while the bulk of the total auxin requires to be released from an inactive state by alkaline hydrolysis. In rye a N/50 NaOH solution gives the optimal effect of alkalinity (Table III).

The auxin of the rye grain is located outside the embryo, in the endosperm and aleurone. Nevertheless the major portion is adjacent to the embryo, being most concentrated in the aleurone layer (Tables IV and V).

The course of auxin production in the developing grain has been followed in three successive summers. Free auxin appears early in the third week after anthesis, accumulates during the next month to a peak value, and with ripening almost disappears (Fig. 1). Alkaline hydrolysis, using a pH 10 buffer,

releases little if any auxin from the inactive form during the period of accumulation, but during ripening the ratio of alkali-extractable to water-extractable auxin increases from about 1 to 20 (Table VII). Total auxin itself decreases to one-seventh of its peak value (Fig. 1). Under optimal conditions of alkalinity, using N/50 NaOH, there is a release of auxin throughout the period of accumulation with a ratio of total auxin to free auxin of about 3, and during ripening the relatively lower loss of total auxin to about one-half the peak value increases this ratio to the order of 80 (Table IX, Fig. 2). Spring rye and winter rye are quite similar in their auxin production.

The effect of premature harvesting on the course of auxin production has been examined. The auxin cycle is of the same kind as occurs during maturation on the plant, a phase of rising auxin content attaining a peak value, followed by disappearance of auxin as the grain loses water and ripens, with the attendant increase in the total/free auxin ratio (Tables XI and XII, Fig. 3). The earlier the harvest the lower the peak value attained but the longer the time from harvesting to the peak value. In harvest as late as 5 weeks after anthesis accumulation must be quickly brought to a standstill, for at the end of a week auxin is in process of disappearing. The loss of total auxin in ripening is relatively less in the earlier harvested ears, with the result that the most dwarfed grain has the highest concentration of auxin (Fig. 4, *a* and *b*).

To ascertain whether auxin trends in the developing grain are local to the grain or involve translocation to and from other regions of the ear, auxin analysis of the whole ear was undertaken from a time prior to emergence, when no auxin is detectable. Auxin first appears in the ear as it emerges, and is at this time confined to the anthers (Table XIV). As these develop and ripen there is a cycle of accumulation and disappearance resembling that later seen in the carpel. Alkaline hydrolysis releases further auxin from the inactive state, but this action is not manifest in ripe pollen as it is in ripe grain. There was no evidence of movement of auxin from anther to carpel and the two sites of auxin production in the ear may be regarded as independent.

Auxin accumulation in the grain cannot be related to the assimilation of dry matter, for the maximum rate of dry-weight increase occurs before any large accumulation of auxin.

The disappearance of total auxin, and the abrupt change in the ratio of total auxin to free auxin seems to be connected with the water loss during ripening, the critical point coinciding with the change in the consistence of the endosperm from the milky to the dough state. Allied with this change is the acquirement of the power of the grain to germinate, probably due to free gaseous exchange consequent on the pericarp becoming permeable to oxygen.

The release of auxin by alkaline hydrolysis has suggested the presence in the grain of a 'precursor', though it is not certain whether a specific inactive substance is concerned, or whether the auxin is released from a bound state. The term 'precursor' is misleading, for there is no evidence to date of its formation previous to the formation of auxin. The suggestion that auxin may be adsorbed on the surface of a protein would account for the constant

auxin-precursor ratio during the phase of accumulation, and possibly the change in ratio once a certain stage in the drying out of the grain is reached. It would not account for the loss in total auxin in the ripening grain.

The function of the auxin stored in the rye endosperm is not certain, but it is evident from the similar auxin contents of spring and winter grain, and the absence of detectable auxin in the embryo during development and also during germination at normal and low temperature, that it is not concerned in the vernalisation process. Cholodny's hormone hypothesis of vernalisation cannot, therefore, be accepted.

In conclusion the author wishes to thank Dr. R. G. Hatton for facilities to carry out the work at East Malling Research Station, Dr. O. N. Purvis for helpful consultation, and Professor F. G. Gregory for many valuable discussions.

LITERATURE CITED

- ARCHBOLD, H. K., 1942: Physiological Studies in Plant Nutrition. XIII. Experiments with Barley on Defoliation and Shading of the Ear in Relation to Sugar and Metabolism. *Ann. Bot., N.S.*, vi. 487-531.
- AVERY, G. S., JR., 1939: Alcohol Extraction of Growth Hormone from Plant Tissue. *Amer. Journ. Bot.*, xxvi. 679-82.
- , BERGER, J., and SHALUCHA, B., 1941: The Total Extraction of Free Auxin and Auxin Precursor from Plant Tissue. *Amer. Journ. Bot.*, xxviii. 596-607.
- , —, 1942: Auxin Content of Maize Kernels during Ontogeny from Plants of Varying Heterotic Vigor. *Amer. Journ. Bot.*, xxix. 765-72.
- , —, 1942 a: Total Auxin Extraction from Wheat. *Amer. Journ. Bot.*, xxix. 612-16.
- , CREIGHTON, H. B., and SHALUCHA, B., 1940: Extraction Methods in Relation to Hormone Content of Maize Endosperms. *Amer. Journ. Bot.*, xxvii. 289-300.
- BERGER, J., and AVERY, G. S. JR., 1944: Isolation of an Auxin Precursor and an Auxin (Indole-acetic Acid) from Maize. *Amer. Journ. Bot.*, xxxi. 199-203.
- , —, 1944 a: Chemical and Physiological Properties of Maize Auxin Precursor. *Amer. Journ. Bot.*, xxxi. 203-8.
- BISHOP, L. R., 1944: Memorandum on Barley Germination. *Journ. Inst. Brewing*, i (xli. N.S.), 166-85.
- BRIGGS, G. E., KIDD, F., and WEST, C., 1920: A Quantitative Analysis of Plant Growth. Pt. I. *Ann. Appl. Biol.*, vii. 103-23.
- , —, 1920 a: A Quantitative Analysis of Plant Growth. Pt. II. *Ann. Appl. Biol.*, vii. 202-23.
- BROWN, H. T., and MORRIS, G. H., 1890: Researches on the Germination of Some of the Gramineae. *Journ. Chem. Soc.*, lvii. 458-528.
- BUY, H. G. DU, 1938: A Method for Extracting Growth Substances from Pigmented Tissues. *Journ. Agric. Res.*, lvi. 155-8.
- CHOLODNY, N. G., 1935: Über das Keimungshormon von Gramineen. *Planta*, xxiii. 289-312.
- , 1936: On the Theory of Yarovisation. *Comptes Rendus Acad. Sci. U.R.S.S.*, iii. 391-4.
- , 1939: The Internal Factors of Flowering. *Herb. Rev.*, vii. 223-47.
- GREGORY, F. G., and PURVIS, O. N., 1936: Vernalisation of Winter Rye during Ripening. *Nature*, cxxxviii. 973.
- , —, 1938: Studies in Vernalisation of Cereals. II. The Vernalisation of Excised Mature Embryos and of Developing Ears. *Ann. Bot., N.S.* ii. 237-51.
- GUSTAFSON, F. G., 1939: The Cause of Natural Parthenocarpy. *Amer. Journ. Bot.*, xxvi. 135-8.
- , 1939 a: Auxin Distribution in Fruits and its Significance in Fruit Development. *Amer. Journ. Bot.*, xxvi. 189-94.

- HAAGEN-SMIT, A. J., LEECH, W. D., and BERGREN, W. R., 1942: The Estimation, Isolation and Identification of Auxins in Plant Materials. *Amer. Journ. Bot.*, xxix, 500-6.
- HATCHER, E. S. J., 1943: Auxin Production during the Development of the Grain in Cereals. *Nature*, cli, 278-9.
- , and GREGORY, F. G., 1941: Auxin Production during Development of the Grain of Cereals. *Nature*, cxlviii, 626.
- KÖGL, F., ERXLEBEN, H., and HAAGEN-SMIT, A. J., 1934: Über die Isolierung der Auxine a und b aus pflanzlichen Materialien. IX. Mitteilung. *Z. physiol. Chem.*, ccxxv, 215-29.
- LAIBACH, F., and MEYER, F., 1935: Über die Schwankungen des Auxingehaltes bei *Zea mays* und *Helianthus annuus* im Verlauf der Ontogenese. *Senckenbergiana*, xvii, 73-86.
- LINK, G. K. K., and EGGERS, V., 1940: Avena Coleoptile Assay of Ether Extracts of Nodules and Roots of Bean, Soybean and Pea. *Bot. Gaz.*, ci, 650-7.
- MURNEEK, A. E., and WITTWER, S. H., 1943: Synapsis and Syngamy as Stimulating Processes of Plant Development. *Science*, xcvi, 384-5.
- NUTMAN, P. S., 1939: Studies in Vernalisation of Cereals. VI. The Anatomical and Cytological Evidence for the Formation of Growth Promoting Substances in the Developing Grain of Rye. *Ann. Bot., N.S.*, iii, 731-57.
- , 1941: Studies in Vernalisation of Cereals. VII. A Study of the Conditions of Formation and Subsequent Growth of Dwarf Embryos of Rye. *Ann. Bot., N.S.*, v, 353-74.
- OVERBEEK, J. VAN, 1938: A Simplified Method for Auxin Extraction. *Proc. Nat. Acad. Sci.*, xxiv, 42-6.
- , 1938a: Auxin Production in Seedlings of Dwarf Maize. *Plant Physiol.*, xiii, 587-98.
- PURVIS, O. N., 1934: An Analysis of the Influence of Temperature during Germination on the Subsequent Development of Certain Winter Cereals and its Relation to the Effect of Length of Day. *Ann. Bot.*, xlviii, 919-55.
- , 1944: Studies in the Vernalisation of Cereals. VIII. The Role of Carbohydrate and Nitrogen Supply in the Vernalisation of Excised Embryos of 'Petkus' Winter Rye. *Ann. Bot., N.S.*, viii, 285-314.
- , and GREGORY, F. G., 1937: Studies in Vernalisation of Cereals. I. A Comparative Study of Vernalisation of Winter Rye by Low Temperature and by Short Days. *Ann. Bot., N.S.*, i, 569-92.
- SKOOG, F., 1937: A Deseeded Avena Test Method for Small Amounts of Auxin and Auxin Precursors. *Journ. Gen. Physiol.*, xx, 311-34.
- THIMANN, K. V., 1934: Studies on the Growth Hormones of Plants. VI. The Distribution of the Growth Substance in Plant Tissues. *Journ. Gen. Physiol.*, xviii, 23-34.
- , and LANE, R. H., 1938: After Effects of the Treatment of Seed with Auxin. *Amer. Journ. Bot.*, xxv, 538-43.
- , SKOOG, F., and BYER, A. C., 1942: The Extraction of Auxin from Plant Tissues. II. *Amer. Journ. Bot.*, xxix, 598-606.
- WENT, F. W., 1928: Wuchsstoff und Wachstum. *Rec. Trav. Bot. Néerl.*, xxv, 1-116.
- , and THIMANN, K. V., 1937: *Phytohormones*. The Macmillan Co., New York. Pp. 294.
- WITTWER, S. H., 1943: Growth Hormone Production during Sexual Reproduction of Higher Plants with Special Reference to Synapsis and Syngamy. *Mo. Agr. Exp. Sta. Res. Bul.* 371. Pp. 58.

Adaptive Isochromosomes in *Nicandra*

BY

C. D. DARLINGTON

AND

E. K. JANAKI-AMMAL

(*John Innes Horticultural Institution, Merton*)

With ten Figures in the Text

I. THE SPECIES

NICANDRA PHYSALOIDES is the solitary species of its genus or even tribe. Like some of its nearest relatives in *Nicotiana* it is a native of Peru and Chile. Like them, too, it has become nearly cosmopolitan in cultivation in the last 200 years. It is widely naturalized in the tropics. It was introduced into gardens in this country in 1759 and is still a favourite botanic garden plant. In cultivation and as an escape four completely inter-fertile 'varieties', or rather variants, can be recognized and we are indebted for them to the following sources:

- (i) *typica*, corresponding to the figure in Curtis's 'Botanical Magazine' (No. 2458): from the Botanic Gardens of Kew (4 plants) and of Montreal (30 plants); and collected for us by Dr. S. C. Harland near Lima (6 plants).
- (ii) *violacea*, having stronger pigmentation of stem, petiole, calyx, leaf-hairs, and seeds than *typica* but intergrading polygenically with it; erroneously described by Lemoine (1906) as a distinct species: from Kew Gardens (10 plants).
- (iii) *immaculata*, lacking the corolla spot and the leaf-hair and cotyledon pigmentation; a recessive single-gene variant, heterozygotes selfed having given us 307 normal to 100 spotless (cf. Dahlgren, 1924): collected in Malabar and from Kew Gardens (22 plants).
- (iv) *alba*, with white instead of blue flowers probably also a recessive single-gene variant: from Cambridge Botanic Gardens (6 plants).

The first three variants were also found as escapes from earlier cultivation at Merton.

2. CHROMOSOME TYPES

We can confirm the original chromosome count (Janaki-Ammal, 1932). All the 68 plants of types (i), (iii), and (iv) examined in 1941 had 20 chromo-

somes in root-tips and flowers.¹ Of the 10 pairs at meiosis, however, one always consists of two isochromosomes. Each of these has two arms with nucleolar constrictions and evidently identical since they are capable of pairing with one another at meiosis. The combination is therefore genetically quadri-

valent and the species has the unique property of being regularly tetrasomic for a part of its chromosomes and disomic for the rest.

The ordinary disomic chromosomes, which we may call autosomes, include yet another pair of nucleolar chromosomes so that there must be in the whole complement of the species six nucleolar organizers.



FIG. 1. Root-tip mitosis in normal plant with two isochromosomes (2BD Flemming, gentian violet). ($\times 6,000$.)

In resting nuclei of the roots about 20 large pieces of heterochromatin always appear and in the pollen grains about 10. These prochromosomes presumably correspond to the centric segments indifferently of all the chromosomes and the isochromosomes cannot be certainly distinguished. They may, however, be entirely heterochromatic. The premeiotic resting nucleus in the anthers shows no heterochromatin.

At meiosis the two isochromosomes, as we shall see, fail to pair with one another in some pollen mother cells and some of these give rise to pollen grains which lack an isochromosome. Such grains probably do not develop and have not been seen in mitosis. Nevertheless over 90 per cent. of the ripe pollen was good in several individuals examined.

In the embryo-sac similar loss occurs (Fig. 4) and we might expect 9-chromosome egg cells to be formed which, being fertilized, would give a proportion of 19-chromosome progeny. What happens to these deficient embryos? The answer to this question was provided by the germination at Merton in 1941 of seed of *violacea* type which had lain in the ground since 1913. Of 8 plants examined only 2 were normal. The remaining 6 were of the missing type with only one isochromosome ($2n = 19$). Turning to a semi-naturalized population at Kew we again found deficient plants, 3 out of 10.

The deficient seedlings did not owe their deficiency to the age of their seed. Six plants raised from packeted 1930 seed were all normal. Their selective appearance was evidently due to their delayed germination without which indeed the strain would have died out at Merton. To test this explanation a germination test was applied. The 68 seedlings originally examined had all been samples selected (as must happen in pot culture even more regularly than in field culture) for rapid germination. Selfed seedlings of one of the 19-chromosome plants were pricked out in batches according to order of germination and one set was further recorded for order of flowering

¹ Pollen mother cells were smeared with acetic lacmoid containing a trace of Bismarck brown (Darlington and La Cour, 1942). Pollen grains were smeared in half-strength iron aceto-carmine.

(Table I). Our expectation was realized. All the early germination was of normal plants; only later did the deficient appear.

Strains vary widely in seed size. Within strains it is inversely proportional to seed number per capsule which varies in normal plants from 400 to 1,000.

TABLE I A

Selfed Progeny of a Plant with 19 Chromosomes, type violacea, with 55.7 per cent. of Full Pollen Grains

Order of germination shown by pricking out; sown 18/2/42. Number of plants counted shown in brackets

Batch pricked out.	Full pollen grains.		
	>90%	50-90%	0-50%
I. 15 days	16(12)	0	0
II. 25 days	15(15)	4(4)	0
III. 32 days	4(2)	2	5
	2n = 20	2n = 19	— ¹

¹ Plants too stunted to examine.

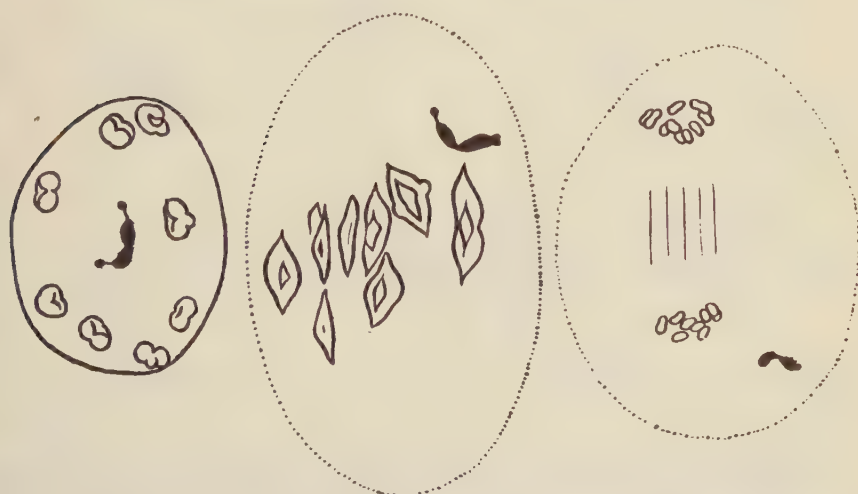


FIG. 2. Diakinesis, first metaphase and anaphase in pollen mother cells of a diploid with one isochromosome (in black). ($\times 2,200$.)

Within capsules seed size is highly uniform. As expected therefore it provided no means of distinction between 19- and 20-chromosome embryos.

The 19-chromosome plants, though often dwarf (Table I B), were not regularly distinguishable from normals in external form. In pollen and egg fertility of course they suffer. Their proportion of full pollen varies from 52 to 56 per cent. Thus half the grains, lacking an isochromosome, die, some of them doubtless too early to figure in the count. One plant recorded as having 19 chromosomes gave 79 per cent. germination, but this must have been due

to the restoration of 20 chromosomes in one anther or follicle by non-disjunction at mitosis.

TABLE I B

Selfed Progeny as in Table I A

Order of flowering; sown 19/12/41

Batch.	$2n = 20$	$2n = 19$
Early germination, <i>normals</i>	0-18	19, 20
Late germination, <i>normals</i>	24, 25	22, 23
<i>dwarfs</i>	—	21, 26, 27

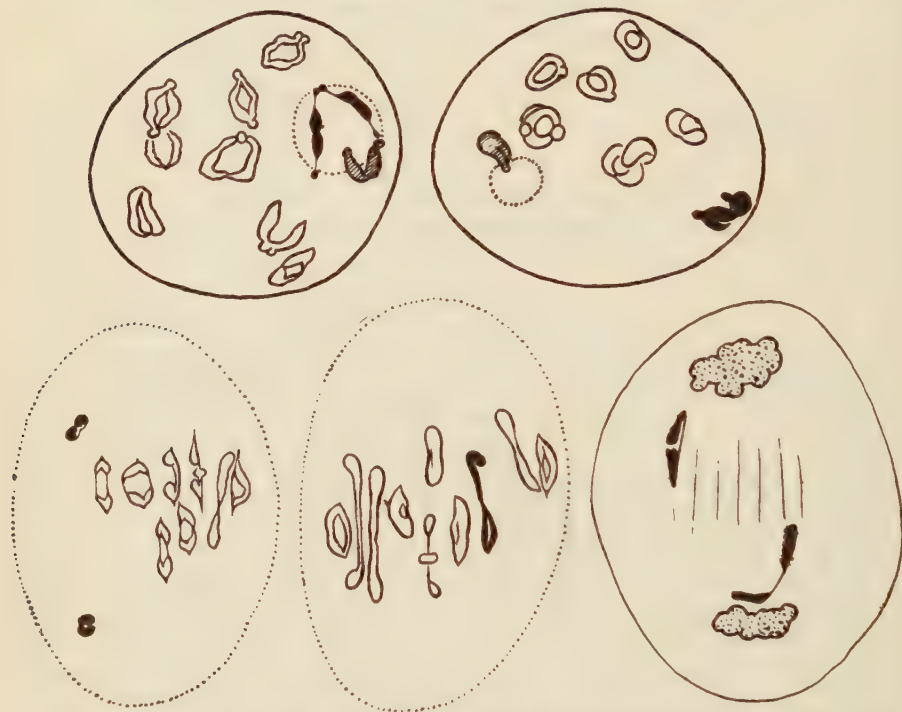


FIG. 3. Diakinesis to first telophase in a diploid with two isochromosomes. The nucleolar autosome pair is hatched. The lagging isochromosomes show attempted misdivision at anaphase. ($\times 2,200$.)

3. THE PRODUCTION OF HAPLOIDS AND POLYPLOIDS

Normal diploids set three capsules of seed with pollen of *Hyoscyamus niger*, and one each with garden *Petunia* and *Nicotiana alata* (all diploids). The seeds were uniformly small, 33 from the first (11 per capsule), 6 from the second, and 20 from the third cross. They developed, however, into normal maternal diploids. It seems likely that haploid parthenogenesis was followed by mitotic recovery as frequently happens in other solanaceous plants when crossed with foreign pollen.

Tetraploids were produced by treating the selfed seedlings of 20-chromosome plants with a 0.2 per cent. aqueous solution of colchicine. A drop of the solution was applied between the two cotyledons. Fifteen out of 33 proved to be tetraploid. Even higher proportions were obtained by wetting the axillary buds after amputation of the older plants.

The 15 plants had the characteristic stouter growth and larger flowers of tetraploids. They could be critically distinguished from diploids by the folding of the corolla in the bud. The ovary and stamens are disproportionately

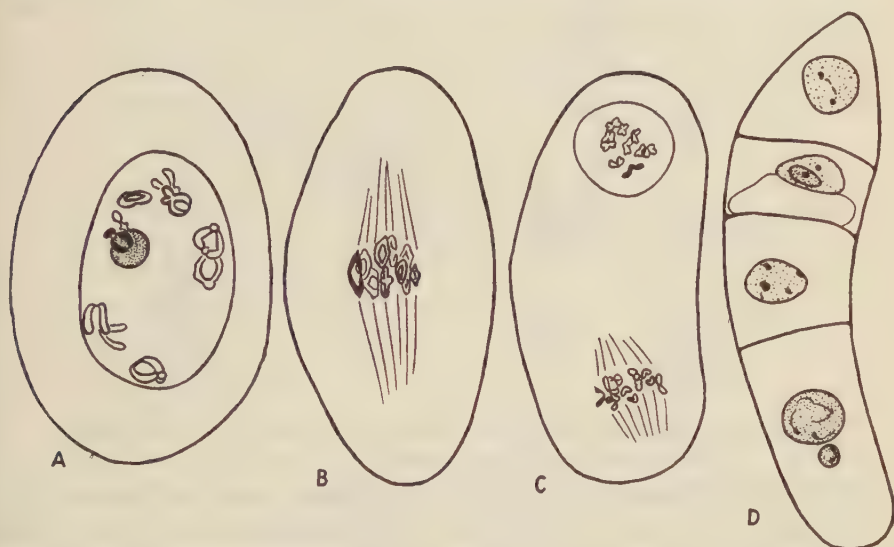


FIG. 4. A—D Diakinesis to second telophase in the embryo-sac mother cell of a diploid with two isochromosomes. An isochromosome has been left out of the main embryo-sac nucleus in D. ($\times 2,200$.)

increased in size so that the corolla is not large enough to cover them completely.

As usual in autotetraploids, the seeds and full pollen grains were twice the volume of those of diploids. As usual also, the pollen and seed fertility was reduced owing to the irregular segregation of quadrivalents at meiosis. Four plants had 52, 58, 67, and 68 per cent. of full pollen grains at maturity. A proportion of giant unreduced pollen grains are formed.

The seed of tetraploids is very variable in size but it begins to germinate more quickly than that of diploids and the selfed progeny is highly uniform in appearance. Eighteen plants examined from early and late germinations all had 40 chromosomes including 4 isochromosomes. About 6 per cent. of seeds, however, fail to throw off the testa and these may have been the ones containing 3 or 5 isochromosomes.

Diploids crossed with tetraploid pollen gave a few apparently good seeds which failed to germinate. The reciprocal cross gave seed of which only

tetraploids germinated. Evidently the tetraploid style had selected unreduced pollen from the diploid (cf. section 6), as it frequently does in such crosses.

Among the selfed progeny of a diploid, 20-chromosome, immaculata from Kew, such unreduced or binucleate pollen gave rise to one triploid. This plant resembled a tetraploid rather than a diploid in external appearance. At meiosis it proved to be partially asynaptic in the pollen mother cells. Less than 5 per cent. of its pollen was full and it was totally seed sterile.

Finding the failure of meiosis in a triploid is not just a coincidence. The

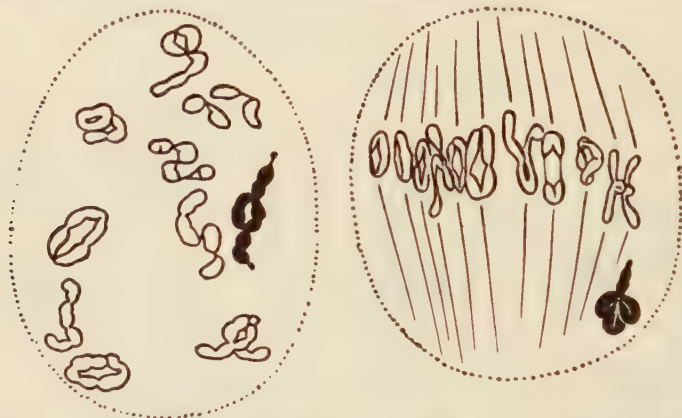


FIG. 5. Diakinesis and first metaphase in the pollen mother cells of a tetraploid with four isochromosomes. ($\times 2,200$.)

combination is favoured in two ways. First, failure of meiosis permits parthenogenesis and is therefore a condition of reproduction in any triploid. This is the case in nature with triploid forms of *Allium amplexans* (Levan, 1940). Secondly, failure of meiosis is likely to be inherited from any parent which itself, as a result of such failure, bore unreduced pollen and hence begot triploid progeny. This is the sequence of events revealed in experiments with *Capsicum* (Ramanujam and Pal, 1940). Our triploid suggests the same history.

4. THE AUTOSOMES AT MEIOSIS

In the diploids with 19 and 20 chromosomes, the autosomes regularly form 9 bivalents with 1 or 2 chiasmata, usually terminalized at metaphase (Figs. 2-4).

In the tetraploids of the second colchicine, or C_2 , generation the frequency and distribution of chiasmata which were recorded were not significantly different from those in the diploid (Table II). Five cells had 42 out of the potential 45 quadrivalents and these were usually of the ring or chain type expected with a low chiasma frequency (Fig. 5).

TABLE II

Chiasmata in Autosomes

Plant.	Cells.	Chrs.	Xta.	X-frequency.
$2x + 1iso$	5	45	79	1.76
$2x + 2iso's$	5	45	80	1.77
$4x + 4iso's$	5	90	164	1.82

5. ISOCHROMOSOMES AT MEIOSIS

Chiasmata in the isochromosomes are either terminal, as in the autosomes, or they lie next to the nucleolar constriction. The centromeres of the isochromosomes, however, must be weaker for, both as bivalents and as quadrivalents, they lag in first metaphase congression and in first anaphase separation. Even at mitosis in root-tips, anthers, and ovaries similar lagging occurs.

At diakinesis the nucleolar constrictions of the isochromosomes are very marked and they even survive at metaphase clearly enough to serve for recognition. A second sign, however, is even more valuable. Failing to congress and lying off the plate, like the sex chromosomes of mammals, they can be readily found and recorded in every cell. It is then seen that the opportunity of

TABLE III

Pairing and Chiasma Formation in Isochromosomes according to Univalent or Bivalent Configuration in 56 P.M.C. of the Normal Diploid ($2x+2iso$'s)

Type of configuration.	I.	II.	Total.
No. of configurations	52	30	82
No. of chromosomes	52	60	112
Xta { within iso's	47	—	47
between iso's	—	28	28
multiples	—	12	12
TOTAL Xta	47	40	87
Xta p. chromosome	0.90	0.67	0.78

N.B. Chiasma frequency is higher with purely inside pairing than with both inside and outside pairing.

TABLE IV

Pairing and Chiasma formation of Isochromosomes according to Configuration in 50 Cells of the Tetraploid (cf. Fig. 6)

Type of configuration.	I.	II.	III.	IV.	Total.
Nos. of configurations	56	21	26	6	—
Nos. of chromosomes	56	42	78	24	200
Xta { within iso's	51	—	2	2	55
between iso's	—	32	61	25	118
multiples	—	4	24	5	33
TOTAL Xta	51	36	87	32	206
Xta p. chromosome	0.91	0.86	1.12	1.33	1.03

TABLE V

Summary of the Pairing of different Numbers of Isochromosomes at Meiosis

Types.	$2x+1 = 19$	$2x+2 = 20$	$4x+4 = 40$
Cells	70	56	50
Chromosomes	70	112	200
Xta { within iso's	56	47	55
between iso's	—	28	118
multiples	—	12	33
TOTAL Xta	56	87	206

TABLE VI

Summary of Chiasma Frequencies in Isochromosomes

Ploidy.	Iso's.	Cells with diff. Nos. of Xta.							Total Xta.	Total Iso's.	Xta p. Chr.
		0	1	2	3	4	5	6			
2x	1	14	56	—	—	—	—	—	56	70	0.80
2x	2	1	25	28	2	—	—	—	87	112	0.78
3x	3	—	—	10	12	13	7	—	143	126	1.13
4x	4	—	—	—	12	22	14	2	206	200	1.03

multiple chiasma formation raises the chiasma frequency in polyploids as compared with diploids (Table VI).

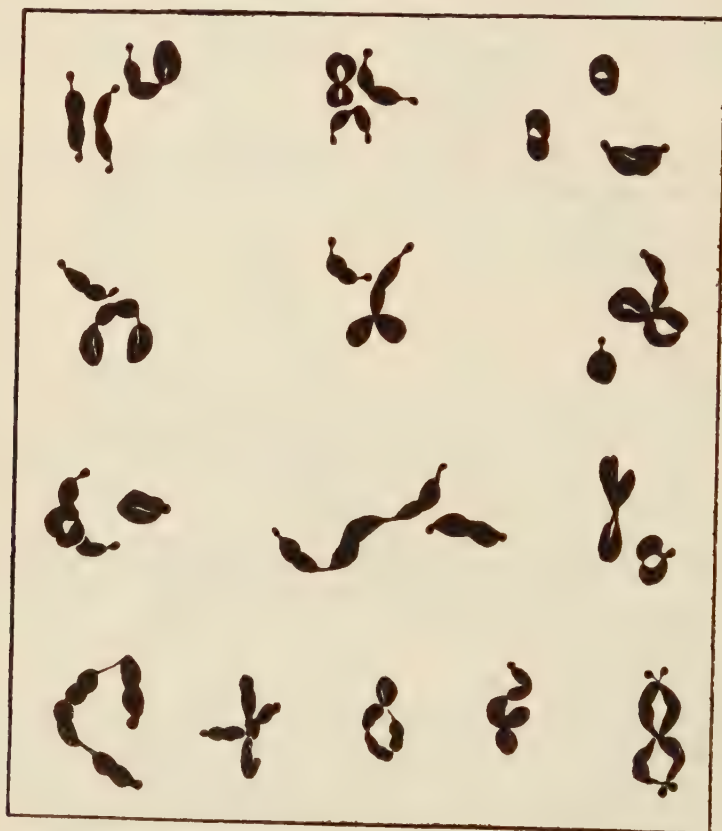


FIG. 6. Configurations of isochromosomes in the tetraploid. ($\times 2,800$.)

The single isochromosome in 19-chromosome plants usually forms a chiasma with itself. This *inside* pairing still has an advantage over *outside* pairing when there are several isochromosomes. Thus the proportions of one to the other expected and found (Tables III, IV, and V) are as follows:

Type.	Observed rates.	Expected.	Advantage factor.
$2x + 2\text{iso's}$	47:28 or 1.68	$\frac{1}{2}$ or 0.50	3.4
$4x + 4\text{iso's}$	55:118 or 0.47	$\frac{1}{4}$ or 0.17	2.8

The position advantage is, as it should be, slightly less in the tetraploid since the chances of outside pairing must increase with the number of iso-

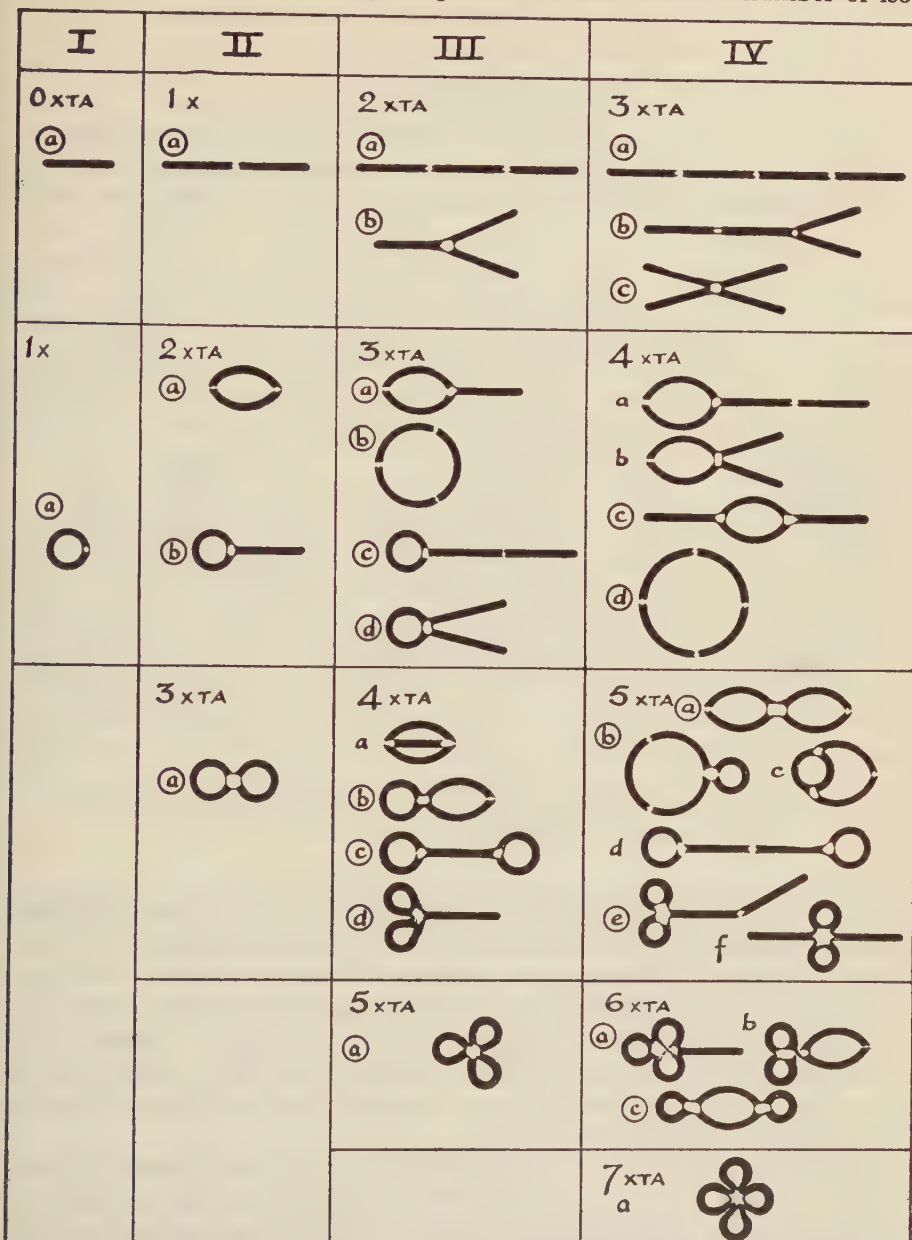


FIG. 7. Diagram showing possible and observed (with letters ringed) configurations of isochromosomes in the tetraploid, according to the numbers of chromosomes and of chiasmata.

chromosomes. This advantage operates against the formation of high configurations, and we find that the trivalent isochromosome is still the most

frequent type even in the tetraploid (Table IV). But its most striking effect appears, as we shall see, only with partial asynapsis.

6. DIFFERENTIAL ASYNAPSIS

Pairing partly fails at meiosis in the pollen mother cells of our spontaneous triploid. This failure provides a critical test of certain conditions of pairing since the two kinds of chromosome differ in regard to these conditions.

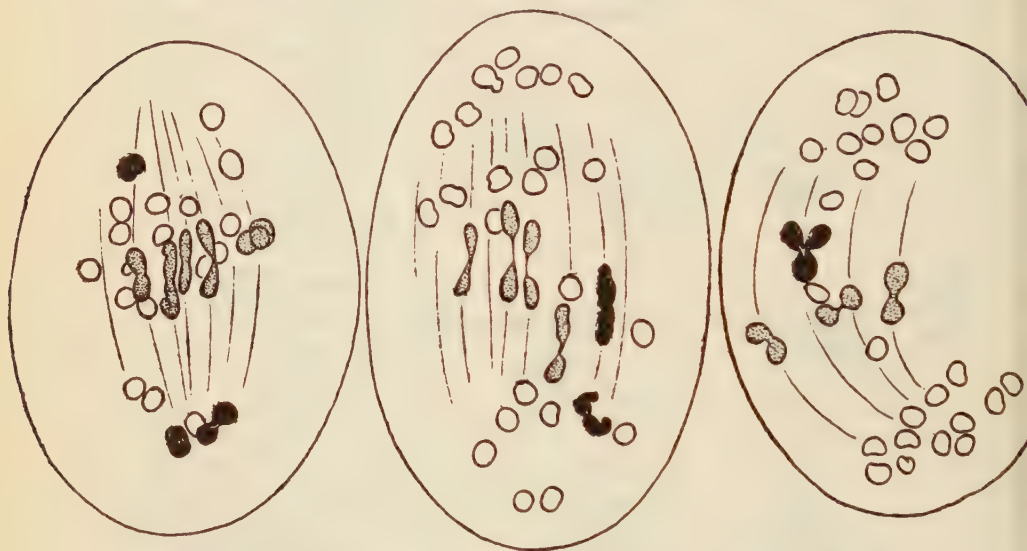


FIG. 8. Abortive first metaphase-anaphase in the triploid with partial asynapsis of autosomes and normal pairing of isochromosomes. ($\times 2,200$.)

The autosomes have to move in order to pair while the isochromosomes have their pairing arms already attached. The reaction of the two types is in fact sharply contrasted. The autosomes, instead of 8 or 9 trivalents, form 3, 4, or 5 bivalents and a rare trivalent. The isochromosomes, on the other hand, have their chiasma frequency unreduced. Rather is it slightly higher in this triploid than in any normal diploid or tetraploid, a property we associate with the trivalent being the most frequent isochromosome configuration even in the tetraploid (Fig. 8 and Table IV).

Asynapsis in this plant is therefore to be attributed to the autosomes having too little time to pair. Prophase is insufficiently precocious and the time limit interrupts pairing before most of the autosomes have come together, although not before the isochromosomes, with their internal advantage of position, have fully united (cf. Frankel et al., 1940). The validity of this explanation could be tested in diploid maize where isochromosomes could be brought together with asynapsis.

7. MITOSIS IN THE POLLEN

Equally in 19- and in 20-chromosome plants the only pollen grain mitoses seen are those with 10 chromosomes (Fig. 9). Evidently pollen lacking the isochromosome dies. Indeed some of it must do so very early since the proportion of empty pollen at this time in the 19-chromosome plants is less than 50 per cent. (Table I and section 2).

Two kinds of abnormalities were found, however, in the pollen of diploids. The first was in an immaculata plant from Kew. It had, in different anthers, 20 to 34 per cent. of giant pollen grains. These had been formed owing to the failure of the second division wall after meiosis, as in *Saccharum* (Janaki-Ammal, 1941) and, for both divisions, in the pear *Beurré Bedford* (Thomas unpubl.). Such pollen would be able to produce an effectively diploid gamete for the fertilization of the egg together with a haploid gamete for the fertilization of the endosperm. Thus an apparent obstacle to the direct production of triploids from the crossing of diploids and tetraploids might be overcome (section 3).

The second kind of abnormality was in a violacea from Merton. In an anther sample with 43 per cent. of post-mitotic grains and 14 per cent. of mitotic, i.e. in the middle of the mitotic phase, 5 of the 15 mitoses observed were second mitoses of both nuclei (Fig. 10). The pollen was not giant and could not therefore have been originally binucleate. These mitoses were thus supernumerary. Their occurrence apparently goes with a lack of differentiation of vegetative and generative nuclei. A similar situation was occasionally found in *Sorghum*, where it was similarly associated with an over-hasty 'polymitosis' and with the presence of supernumerary chromosomes (Darlington and Thomas, 1941).

TABLE VII

Chromosome Numbers at Mitosis in Pollen Grains of violacea Tetraploids (4x+4iso's) from Samples differing in the Phase of Development

Plant.	Binucleate Grains.	Chromosome Numbers.			Total.
		18	19	20	
K ₂ a ₂	28%	—	6	16	22
K ₂ a ₁	28%	1	7	12	20
K ₂ a ₁	83%	5	2	4	11

In the tetraploids there is a range of chromosome numbers which depends on the presence of 17 or 18 autosomes and 1 or 2 isochromosomes. In order to find out whether a deficiency of either type was deleterious we compared the frequencies of deficiencies at different mitotic phases (cf. Darlington and La Cour, 1945). The observations showed that the defective pollen, like the defective seeds of the diploid, developed more slowly than the normal (Table VII).



FIG. 9. Pollen grain mitoses of a diploid (above) and of a tetraploid (below) with different numbers of isochromosomes and autosomes. ($\times 2,200$.)

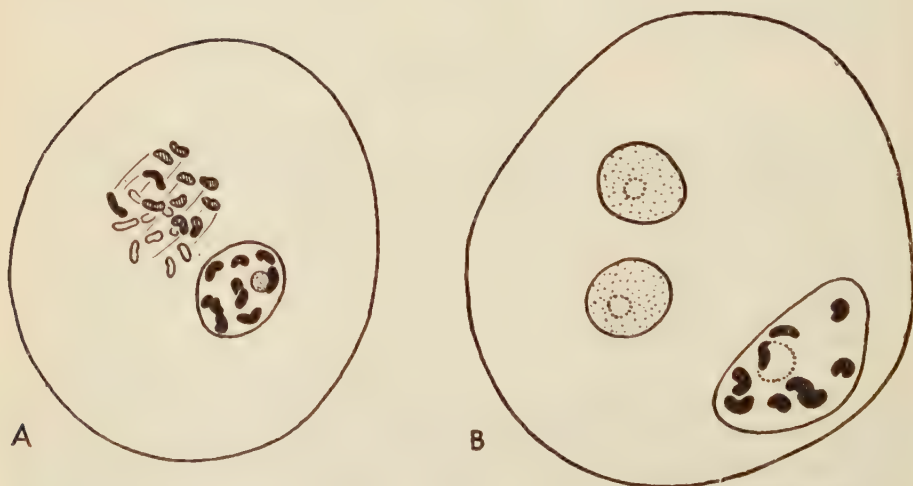


FIG. 10. Two stages of polymitosis in pollen of a diploid with undifferentiated nuclei. ($\times 2,200$.)

8. ISOCHROMOSOMES IN EQUILIBRIUM

Without undue hazard we may assume that the isochromosomes of *Nicandra* have arisen from ordinary chromosomes of the same permanent type as its autosomes. Such an origin of isochromosomes is now fairly well understood elsewhere (Darlington, 1940; Rhoades, 1940; Giles, 1943; Darlington and La Cour, 1944). Misdivision of the centromere, of an ordinary two-armed chromosome at meiosis is due either to failure of pairing (seen in *Tulipa*, *Pisum*, and here in *Nicandra* and to be inferred in *Datura* and *Zea Mays*) or to a failure of co-orientation of any chromosome (*Fritillaria*) or only of the longest (*Gasteria*). By misdivision telocentric chromosomes arise which at once or later undergo sister reunion of chromatids within the centromere and thus turn themselves into isochromosomes.

Isochromosomes have been found in exceptional stocks not only in these plants but also in *Sorghum* (Darlington and Thomas, 1941), *Campanula* (Darlington and La Cour, unpubl.), and, we may suppose, as the attached-X chromosomes of *Drosophila*. In none of these, however, does the isochromosome show the evidence of a long past or the promise of a long future. On the contrary where an established chromosome, the Y of *Drosophila melanogaster*, shows signs of having arisen from an isochromosome it also shows signs of the impermanence of its isogenetic character. It has lost most of one arm.

What then are we to assume of the isochromosomes in *Nicandra*? At meiosis they show signs of the abnormal centromere that we expect from an origin by misdivision. The end segments, between which crossing over takes place, must be identical. The correspondence in position of the two nucleolar organizers shows that they are included in these segments. The equal length of the two arms, however, goes farther: it suggests that their original identity has been preserved—preserved, that is, throughout the species and over a great space of time. Such an identity would not preclude the possibility of differentiation between the isochromosomes of different strains. This possibility might seem even more important since the isochromosomes are likely to undergo reconstruction following misdivision (Fig. 2). Yet there are no differences of behaviour between strains and there is therefore little likelihood of irregularity in crosses between strains.

Our problem is thus to find out why chromosomes which in themselves are peculiarly unstable, nevertheless, as part of the heredity of the species both in nature and in cultivation, are peculiarly stable. To do this we must discover what use the special properties of these chromosomes serve in the individual and in the species.

In the individual we see that they are uniformly necessary; that their optimum dose is the normal fourfold dose found in the diploid; but that a twofold dose gives viable results. This condition points to two analogies. The first is with the X-chromosomes in *Orthoptera* and elsewhere, and implies the action of Muller's principle (1932) of dosage compensation with the dosage

rates of two-and-four instead of one-and-two. The second analogy is with the supernumerary but profitable fragments of maize and Sorghum. These, however, can exist in any dosage proportion to the autosomes from nil to equality or beyond. They are non-specific and heterochromatic.

Taking both these analogies into consideration we must regard the isochromosomes of *Nicandra* as adapted to a special function. This function depends, however, on the advantage of the species rather than of the individual. It is that the errors of segregation of the isochromosomes lead to a heterogeneous progeny from homozygous individuals and in homogeneous strains. The mutants, however, have the faculty of reverting to type: 20 gives 19 and 19 gives 20 again. The heterogeneity affects particularly the rate of germination and thus enables a single homozygous plant to distribute its progeny over a greater number of years than it otherwise could. It can spread itself in time as well as in space. Such a property, analogous to that of 'hard seeds' in the Leguminosae, might well confer an advantage of decisive value to a species like *Nicandra physaloides* which is an isolated relic. The lower vitality and delayed germination of the deficient individuals will increase, in the right conditions, the chance of survival of the species while reducing that of a part of the individuals.

The condition of *Nicandra* is at present unique in our knowledge. We must not exclude the possibility, however, that isochromosome formation is a frequent process in nature even though it is rarely stabilized. Chromosomes with defective centromeres (such as the long chromosomes of *Gasteria*) may often have been derived from isochromosomes by loss, as the Y in *Drosophila* appears to have been. Species may use isochromosome formation as a recurring means of genetic reconstruction. Such a reconstruction must be intermediate in its effect on balance between the reduplication of minor segments and secondary polyploidy. Like them it provides a universally available means of achieving a necessary adaptation to a sudden change of the environment.

SUMMARY

1. *Nicandra physaloides* in all its varieties normally has 9 pairs of autosomes and 1 pair of isochromosomes ($2n = 20$).
2. At meiosis the isochromosomes pair either inside (to give univalents) or outside (to give bivalents) or both. When univalents are lost, pollen and eggs are formed lacking an isochromosome altogether.
3. The deficient pollen dies, but the deficient eggs must be fertilized since seedlings of delayed germination include a proportion lacking one isochromosome ($2n = 19$).
4. Slight polymitosis of the pollen occurred in one diploid and binucleate pollen in another.
5. Tetraploids with 4 isochromosomes ($2n = 40$) show the expected configurations and chiasma frequencies. The isochromosomes, as in diploids,

show an advantage of pairing inside over outside. The attachment of the arms thus facilitates inside pairing.

6. A triploid had asynapsis of the autosomes with normal chiasma formation in the isochromosomes. This is attributed to an accentuation in the advantage of attached arms as partners where there is a time limit to pairing.

7. The value of isochromosomes to *Nicandra* as of B chromosomes to maize is in securing heterogeneity. But in *Nicandra* this is a means of survival of the species rather than of the individual for the delayed germination which reduces the survival value of the deficient individuals increases the survival value of the species producing them.

LITERATURE CITED

- BELLING, J., 1927: The Attachment of Chromosomes at the Reduction Division in Flowering Plants. *Journ. Genet.*, xviii. 177-205.
- DAHLGREN, O. K. V., 1924: Kreuzungskleinigkeiten. *Hereditas*, v. 222-30.
- DARLINGTON, C. D., 1940: The Origin of Isochromosomes. *Journ. Genet.*, xxxix. 351-61.
- and LA COUR, L. F., 1942: The Handling of Chromosomes. Allen & Unwin, London.
- , 1945: X-ray Breakage and the Nucleic Acid Metabolism. *Journ. Genet.*, xli. 180-267.
- and THOMAS, P. T., 1941: Morbid Mitosis and the Activity of Inert Chromosomes in Sorghum. *Proc. Roy. Soc. B*, cxxx. 127-50.
- and UPCOTT, M. B., 1941: The Activity of Inert Chromosomes in *Zea Mays*. *Journ. Genet.*, xli. 275-96.
- FRANKEL, O. H., DARLINGTON, C. D., and LA COUR, L. F., 1940: The Causal Sequence of Meiosis I. *Journ. Genet.*, xli. 9-64.
- GILES, N., 1943: The Origin of Isochromosomes at Meiosis (*Gasteria*). *Genetics*, xxviii. 512-24.
- JANAKI-AMMAL, E. K., 1932: Chromosome Studies in *Nicandra physaloides*. *Cellule*, xli. 89-110.
- , 1941: The Breakdown of Meiosis in a Male-sterile *Saccharum*. *Ann. Bot., N.S.*, v. 83-8.
- LEMOINE, H., 1906: *Nicandra violacea*. *Rev. Horticole*, 1906, 208-9.
- LEVAN, A., 1940: The Cytology of *Allium amplexans*, &c. *Her.*, xxvi. 353-94.
- LEWIS, D., 1944: The Incompatibility Sieve for Producing Polyploids. *Journ. Genet.*, xlv. 261-4.
- MULLER, H. J., 1932: Further Studies on the Nature and Causes of Gene Mutations. *Proc. 6th Int. Cong. Gen.* Ithaca, 213-55.
- RAMANUJAM, S., and PAL, B. P., 1940: Asynapsis in Chilli (*Capsicum annum*). *Curr. Sci.*, iii. 126-7.
- RHOADES, M. M., 1940: Studies of a Telocentric Chromosome in Maize, &c. *Genetics*, xxv. 483-520.

Plant-Injection Methods for the Diagnosis of Mineral Deficiencies in Tobacco and Soya Bean¹

BY

B. N. LAL

(East Malling Research Station, Kent)

With Plate III and eight Figures in the Text

INTRODUCTION

THIS communication describes the injection methods (Roach, 1938, 1939) found most suitable for diagnosing mineral deficiencies in the tobacco and soya bean and for other physiological purposes. Preliminary experiments were carried out with dye solutions, and later the methods giving the most useful types of distribution of dye were used for injecting solutions containing compounds of nitrogen, phosphorus, potassium, magnesium, and iron respectively into plants grown in sand supplied with all essential elements except the one injected.

TOBACCO

Methods of cultivation.

The plants were grown in sand culture with known mineral deficiencies in ordinary earthenware pots that had been cleaned, heated, and dipped into almost boiling paraffin-wax until impregnated.

Deficiency symptoms.

(i) *Nitrogen*. Stunted growth, small yellow leaves, and considerable reduction in the number of leaves were the symptoms of nitrogen-starved plants.

(ii) *Phosphorus*. Poor growth of plants and small thin sickly leaves of purplish colour characterized this group.

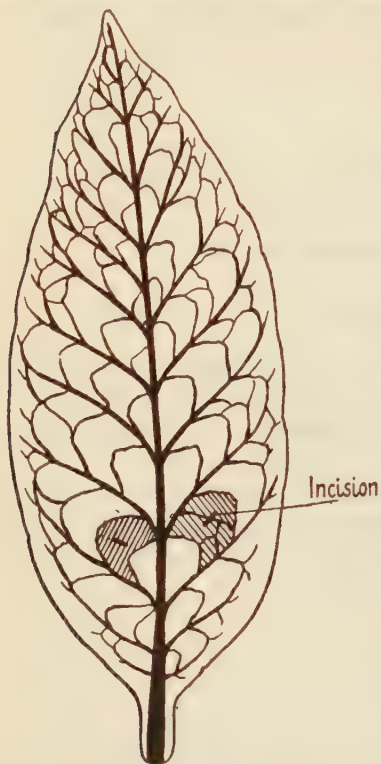
(iii) *Potassium*. Little growth and small, thin, abnormally dark bluish-green leaves were characteristic. They were curled downwards and were limp. Scorching started at the tips of the leaves and gradually extended along the margins.

¹ Part of a thesis approved for Degree of Doctor of Philosophy, University of London, 1939.

At the request of Dr. Lal, who has now returned to India, this paper has been seen through the press by Dr. W. A. Roach, who takes responsibility for certain abridgements and alterations in presentation.

(iv) *Magnesium*. The lower leaves were the first to show deficiency symptoms, the tips turning pale green in the initial stage and becoming chlorotic later.

(v) *Iron*. Complete chlorosis including the veins was symptomatic, growth being finally inhibited.



TEXT-FIG. 1. The distribution of dye resulting from the interveinal injection of a tobacco leaf. Permeated areas are shaded.

Interveinal leaf injection.

Experiments with dyes. The typical distribution pattern by this method (see Roach, 1938, 1939) is shown in Text-fig. 1. The dye moved from the incision both towards the margin and also towards the midrib. On account of the large size of interveinal areas the tubes were usually empty in an hour and a half; they were refilled and replaced in the incision. The area permeated, even after 5 hours, was small, as shown in Text-fig. 1. The dye did not cross the midrib or either of the secondary veins. Although the colour of the permeated area deepened the dye did not make further progress as a result of the second injection.

This type of distribution within the boundary of midrib and secondary veins resembles that obtained by Roach in the apple.

Leaf-tip injection.

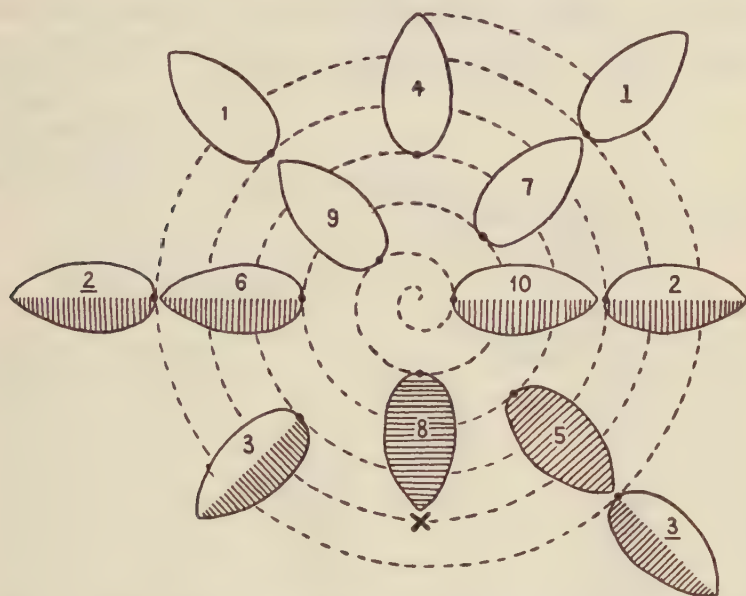
Experiments with dyes. This method consists in cutting off the leaf tip and bending the leaf so that the cut end dips into a tube containing the liquid. The stem was negatively geotropic to a marked degree and there was a tendency for the immersed leaf to be drawn out of the solution. The liquid travelled backwards along the leaf towards the main stem, but if not more than a quarter of the leaf was removed the dye did not travel beyond that leaf.

Leaf-stalk injection.

(a) *Experiments with dyes.* The dye injected by this method produced a definite colouration of the leaves within a few minutes; and within half an hour the distribution could be mapped. During the following hours there was no further spread of the dye, but only an increased intensity of colouration.

The resulting distribution is shown diagrammatically in Text-fig. 2, which is drawn as if the leaves had been detached from the shoot and arranged on a phyllotaxis diagram. Phyllotaxis in tobacco is $\frac{3}{8}$. The results are also expressed in a different form in Table I.

It will be seen from Text-fig. 2 that the dye was distributed amongst leaves in a regular manner. Certain leaves were quite free from dye, others



TEXT-FIG. 2. Distribution of dye resulting from the injection of a tobacco plant by the leaf-stalk method. Injection made through leaf stalk X. Permeated areas shaded.

were coloured on only one side of the midrib, while the rest were completely permeated by the dye. When only one-half of the leaf was permeated the midrib formed a sharp line of demarcation except at the apex. The fraction of the area permeated depended upon its angular distance from the injected leaf-stalk, as observed by Roach in the apple. The less the angular distance of the leaf from the injected midrib the more completely was it permeated, and vice versa. Leaves +4 and -4, four up and four down the spiral respectively, being exactly opposite the injected midrib, were not permeated. Leaves +1 and -1 which were distant $\frac{3}{8}$ circumference from the injected midrib were not permeated at all. Leaves +2 and -2, distant only $\frac{1}{4}$ circumference, were coloured on the halves nearer the point of injection; and so were leaves +16 and +10. Leaves +3 and -3, distant only $\frac{1}{8}$ circumference, were coloured on their halves nearer the injection point and in certain cases, though not usually, the dye entered the distal half of the leaf at the tip. Leaves +5 and -5, again only $\frac{1}{8}$ distant, were completely permeated. The dye permeated first the proximal halves and then spread round the tip to the distal halves

also. Leaf +8, being directly above the injected midrib, was permeated throughout its whole area.

This type of distribution, however, differs in one respect from that in the

TABLE I

Area of Leaves permeated by Dye Solution injected through a Leaf Stalk

(X = injected midrib; + = leaves above one injected; — = leaves below one injected.)

Leaf No.	Area of leaf permeated with dye.	Angular distance (fraction of circumference) from injected midrib.
+11	Complete (young leaf)	$\frac{1}{8}$
+10	One half only	$\frac{1}{4}$
+9	None	$\frac{3}{8}$
+8	Complete	0
+7	None	$\frac{3}{8}$
+6	Slightly less than half	$\frac{1}{4}$
+5	Nearly whole	$\frac{1}{8}$
+4	None	$\frac{1}{4}$
+3	$\frac{1}{2}$ only (sometimes more)	$\frac{1}{8}$
+2	$\frac{1}{2}$ only	$\frac{1}{4}$
+1	None	$\frac{3}{8}$
X		
—1	None	$\frac{3}{8}$
—2	$\frac{1}{2}$ only	$\frac{1}{4}$
—3	$\frac{1}{2}$ only	$\frac{1}{8}$
—4	None	$\frac{1}{4}$
—5	Nearly whole	$\frac{1}{8}$

apple shoot. The number of individual leaves permeated in tobacco is greater than that in apple, even though the phyllotaxis is the same in both. A similar difference has also been noticed between the raspberry (Roach, 1938, 1939) and the potato (Hill and Roach, 1940), which also have the same phyllotaxis ($\frac{3}{8}$).

Roach explained this difference on the basis of the number of conducting strands supplying each leaf and the area of the circumference they cover. For example, the apple leaf-stalk has only one main conducting strand, whereas the raspberry leaf has three, and they are so wide apart that they envelop $\frac{3}{8}$ circumference of the stem when they enter a leaf stalk.

(b) *Experiments with mineral nutrients.* The next step was to inject into plants grown in sand and provided with all but one of the essential elements a solution containing the missing element, and to study the responses of the plant. The duration of injection was 24 hours unless otherwise stated.

(i) *Nitrogen.* Plants deprived of boron but otherwise completely nourished (none of which were showing any sign of boron shortage) were later deprived of nitrogen. These plants soon showed nitrogen-deficiency symptoms and

they provided better material for studying response to nitrogen injection than those deprived of nitrogen from the beginning. Solutions containing 0.25 per cent. of ammonium nitrate and 0.25, 0.5, and 2 per cent. of urea respectively were used for the experiments. The permeated parts of leaves became noticeably greener in about 6 days and the green became more intense for about a further week. The permeated areas also grew more rapidly (see Pl. III, Fig. 1). The response is recorded in Table II.

Leaves on plants deprived of nitrogen from the beginning were few in number, hence the observations could not be extended beyond leaf +5. Out of these only +3 and +4 leaves responded with any certainty.

TABLE II

Area of Leaves responding to Injection of NH_4NO_3 and of Urea

1 = Plants nitrogen-starved from the beginning.

2 = Plants changed to nitrogen-starvation from boron series.

* = leaves damaged (see text).

Leaf No.	NH_4NO_3 (0.25 %)	Urea (%)			
		0.25		0.25	
+8	1	1	2	2	1
+7	—	—	—	Whole*	—
+6	?	Young	—	?	—
+5	?	Young	—	$\frac{1}{2}$	—
+4	Whole	Whole	Whole	Whole	Whole*
+3	$\frac{1}{2}$	$\frac{1}{2}$ slight	$\frac{1}{2}$	$\frac{1}{2}$ *	$\frac{1}{2}$ *
+2	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$ *
+1	None	None	None	None	None

The results of the first two columns suggest that urea and nitrate were equally effective as sources of nitrogen. It will be noticed that one-half of leaf 4 responded to the injection of the nitrogenous solutions, though it had not been penetrated by dye.

A 2 per cent. solution was injected into a plant of series 1 (originally nitrogen-starved) and 0.5 per cent. into a plant of series 2 (changed from boron series). The leaf tip and margin first became flaccid and later turned brown; the damage then spread from the margin to between the veins. The 'scorching' was conspicuous the 3rd day after injection.

From Table II it will be seen that the areas which were benefited by the dilute solution corresponded in position to the areas damaged by the stronger solution.

(ii) *Phosphorus*. Disodium hydrogen phosphate solution (0.25 per cent.) was injected. The permeated halves did not turn perfectly green, as those injected with nitrogenous compounds, but there was such a definite improvement that the permeated halves could be easily recognized. The contrast in the colour of permeated and untreated halves persisted for 4 weeks, whereas the visible effects of nitrogen and potassium tended to fade after 2 to $2\frac{1}{2}$ weeks.

The permeated halves of leaves 3 and 4 became darker green as a result of the injection, but there was no significant growth increase in any of the leaves.

(iii) *Potassium*. The areas responding to leaf-stalk injections of solutions containing potassium are given in Table III.

TABLE III

Area of Leaf responding to Injection of Potassium Salts

Leaf No.	KNO ₃ (0.25%)	KCL + K ₂ SO ₄ (0.25%)
+8	Whole	Whole
+7	None	None
+6	$\frac{1}{2}$	$\frac{1}{2}$
+5	Whole	Whole
+4	$\frac{1}{2}$	$\frac{1}{2}$
+3	$\frac{1}{2}$	$\frac{1}{2}$
+2	$\frac{1}{2}$	$\frac{1}{2}$
+1	None	None
X		

The first response appeared on the 8th day after injection of potassium sulphate-chloride mixture, when the affected areas became greener than the remainder, which still showed deficiency symptoms. There was no visible response to potassium nitrate until the 13th day. The effect of both lasted from 1 to 1½ weeks, after the responses were well defined. The areas responding were identical with those permeated by dye, with the exception of leaf +4, which responded to potassium but was not permeated by dye.

Certainty of diagnosis. To determine to what extent the change in colour was due to injection of potassium and to what extent it could have been the result of natural variation, 100 leaves chosen at random from different plants were examined. Only a single leaf was found having one side different from the other, and in this the difference was slight. An increase in greenness in two or three half-leaves at the end of a predictable length of time after injection, in such a manner as to fill an accurately predictable pattern, could, therefore, only be the result of injection, and the chances of its resulting from chance variation are infinitely remote.

It also became apparent that the permeated halves of leaves not only became greener than the untreated halves but also increased more rapidly in area, with the result that in some there was a little puckering of the lamina (see Pl. III, Fig. 1). In a number of leaves the permeated half was as much as 0.4 cm. wider than the untreated.

(iv) *Magnesium*. Magnesium chloride solution (0.25 per cent.) and magnesium sulphate (0.25 per cent.) were used, and produced identical responses, which are summarized in Table IV.

Every permeated half-leaf was greener and most were broader than the corresponding untreated halves. Greenness began to fade 21 days after injection, but the increase in width was permanent.

TABLE IV
Response to Injection of Magnesium Salts

Leaf No.	Area of leaf improved in colour	
	MgCl ₂	MgSO ₄
+8	Whole	Whole
+7	None	None
+6	$\frac{1}{2}$	$\frac{1}{2}$
+5	Whole	Whole
+4	$\frac{1}{2}$	$\frac{1}{2}$
+3	$\frac{1}{2}$	$\frac{1}{2}$
+2	$\frac{1}{2}$	$\frac{1}{2}$
+1	None	None

(v) *Iron*. Iron citrate (0.025 per cent.) and iron sulphate (0.025 per cent.) were injected, and the responses are summarized in Table V.

The results are again as expected in leaf +2. Leaves +3 and +4 showed a marked response, which appeared on the 6th day after injection and lasted for 3 weeks; during this period the colour difference at first increased and then gradually disappeared. Again the permeated areas grew more than the corresponding untreated ones.

TABLE V
Response to Iron Injection

Leaf No.	Iron citrate	Iron sulphate
+7	?	?
+6	?	None
+5	Whole	Whole
+4	$\frac{1}{2}$	$\frac{1}{2}$
+3	$\frac{1}{2}$	$\frac{1}{2}$
+2	?	?
+1	None	None

The use for diagnostic purposes of growth increase resulting from injection.

Usually three main criteria of response to mineral injection are recognized: (i) improvement in the green colour of the leaf, (ii) prevention of a deficiency symptom, and (iii) cure of an already existing one. The smooth outline, the regular shape and symmetry of the tobacco leaf, made accurate width measurements possible. Injection of potassium, magnesium, and iron was found to have brought about easily detectable increases in width in the permeated as compared with the corresponding unaffected halves.

The effect on leaf colour increased to a maximum, then decreased until it finally disappeared. This may be connected with the fact that elements like potassium and magnesium migrate. The difference in width between the permeated and unaffected halves, however, did not decrease, but could be recognized for practically the whole of the plant's life. Increase in width can

therefore be used as a fourth criterion of response to injection of at least potassium, magnesium, and iron in tobacco.

SOYA BEAN

Methods of cultivation.

In view of the fact that tobacco plants, which had received a complete nutrient for a time before being deprived of the element under experiment, gave better responses than those deprived from the first of the element to be injected, it was decided to give all soya-bean plants, except those intended for minor element experiments, a complete nutrient for the first 2 or 3 weeks. The plants were grown in thoroughly washed sand, watered with suitable nutrient solutions.

The plants deprived of magnesium showed no deficiency symptoms, and injection of solutions of magnesium salts produced no visible response. No further reference is therefore made to the work on magnesium.

Deficiency symptoms.

(i) *Nitrogen*. Plants deprived of nitrogen were the first to show symptoms. These appeared a week after the element was withheld, and 2 days later the plants looked distinctly unhealthy. They bore small yellow leaves, were stunted, and shed their leaves prematurely.

(ii) *Phosphorus*. The young plants of this series were characterized by the slaty appearance of their leaves, which later took on a purplish green colour, and growth was stunted.

(iii) *Potassium*. The plants deprived of potassium bore leaves which had dead areas along their margins.

(iv) *Iron*. Symptoms of iron deficiency appeared a little later than those of nitrogen. Both young and mature leaves became chlorotic.

(v) *Boron*. Boron deficient plants were characterized by the abnormal appearance of the leaves, loss of turgidity, death of growing-points, and tillering. Young leaves presented a finely mottled appearance, the interveinal areas being sometimes almost devoid of chlorophyll (Pl. III, Fig. 4). In some leaves only narrow strips near the veins remained green. Leaves tended to become greener with increasing age and the mature leaf had a mottled yellowish-green appearance. The growth of the plant was stunted.

Interveinal leaf injection.

(a) *With dyes*. A typical result of interveinal injection is shown in Text-fig. 3. The dye moved in all directions from the injection incision, but it remained mainly within the interveinal area after 24 hours. It escaped a little, however, across the thin end of the vein near the leaf margin. It did not cross the midrib nor the lower regions of the two nearest secondary veins.

(b) *With nutrients*. (i) *Nitrogen*. The 5th day after the injection of 0.2 per cent. urea solution each incision was surrounded by an area of a darker green than the remainder of the leaf (Pl. III, Fig. 2). The colour became more

intense and the area of response more extensive, but was confined to the interveinal area except at the upper thinner end of one of the two nearest secondary veins. This response was visible for 1 week, after which the distinction between treated and untreated areas disappeared.

Similar responses were obtained with 0.25 per cent. ammonium nitrate and 0.3 per cent. asparagine; except that with ammonium nitrate there was no detectable response until the 8th day.

(ii) *Phosphorus*. No response was obtained with half-grown leaves. Younger ones were not tested.

(iii) *Potassium*. No detectable response was obtained to injections of solutions of potassium salts.

(iv) *Iron*. As soon as chlorosis was evident, a few interveinal injections of 0.025 per cent. ferrous sulphate were made. A detectable increase in greenness was observed around the incision on the 2nd day after injection; and this was most striking on the 3rd day (see Pl. III, Fig. 3).

This is the quickest response obtained so far by this method. The next quickest response was recorded by Roach (1938, 1939); the injection of 0.05 per cent. iron citrate into a chlorotic apple leaf produced a slight but quite definite green tint in 3 days.

(v) *Boron*. The result of injecting 0.001 per cent. boric acid is seen in Pl. III, Fig. 5. Most injected areas started turning green 7 days after injection, and the resulting greenness lasted for 2-3 weeks. The uninjected areas on the same leaf remained mottled.

Since deficiencies of zinc and iron produce somewhat similar mottlings, 0.01 per cent. zinc sulphate and 0.025 per cent. ferrous sulphate were each injected into boron deficient leaves. Both were without visible effect.

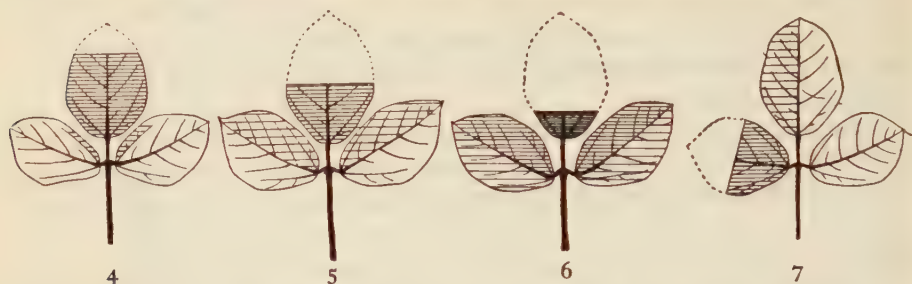
Leaflet-tip method.

(a) *With dyes*. When one-quarter or a little less of the terminal leaflet was removed and the cut end immersed in dye solution, the dye permeated the whole of that leaflet and appeared in minute quantities in only a fraction of the adjacent halves of the other two leaflets, as shown in Text-fig. 4. When about one-half of the terminal leaflet was removed, the whole of the immersed leaflet and adjacent halves of lateral leaflets were completely permeated, as shown in Text-fig. 5. No dye passed beyond the midrib of either lateral leaflet. When about three-quarters of the terminal leaflet was cut off, the lateral leaflets also became completely permeated and their two sides were of about equal intensity (see Text-fig. 6). Injection through a lateral leaflet after half had been removed resulted in the permeation of the proximal half of the terminal leaflet (see Text-fig. 7). The greater symmetry of the terminal leaflet makes this the most useful type of leaflet injection.



TEXT-FIG. 3. Distribution resulting from the interveinal injection of dye solution into a soya-bean leaf. Permeated areas are shaded.

(b) *With nutrients.* (i) *Nitrogen.* About one-half of a terminal leaflet was removed and the attached half was dipped into 0.2 per cent. solution of urea for 24 hours. On the 5th day the immersed leaflet and the proximal halves of the lateral leaflets began to turn green. The permeated halves of the lateral



TEXT-FIGS. 4-7. Distribution resulting from the injection of dye solution through a cut leaflet tip. Fig. 5 shows the result of injecting through a cut lateral leaflet. Figs. 4-6 show the result of cutting off increasing fractions of the terminal leaflet.

leaflets contrasted strikingly with the other halves and with other leaves on the plant, all of which were yellow. The immersed terminal leaflet was of an even more intense green (see Pl. III, Fig. 6).

Five days after three-quarters of a leaflet on another leaf had been removed, and the remainder injected with urea, the terminal, and practically the whole of the lateral, leaflets turned green.

(ii) *Phosphorus.* No definite response resulted from the injection of 0.2 per cent. sodium dihydrogen phosphate through a half leaflet, but the whole leaf was on several occasions slightly greener than the uninjected ones.

(iii) *Potassium.* There was no obvious response to leaflet-tip injections of solutions of potassium salts.

(iv) *Iron.* The part of the leaflet immersed in the ferrous sulphate solution invariably became bleached and was practically dead. More dilute solutions were tried but not sufficiently dilute to be harmless. There was no response in adjacent parts.

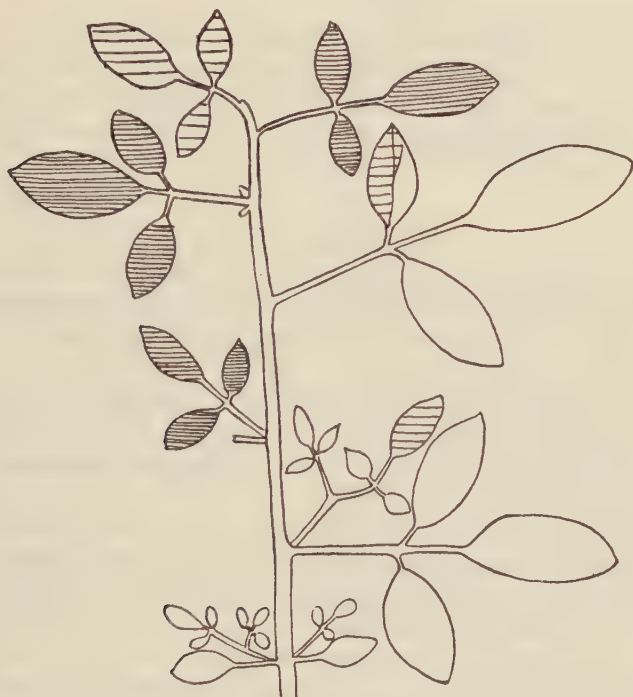
Leaf-stalk injection.

(a) *With dyes.* Patent blue solution (0.25 per cent.) injected by this method soon produced an obvious coloration of neighbouring leaves. The typical final distribution of dye is shown in Text-fig. 8. The dye permeated almost the whole plant above the point of injection. The leaf on the shoot in the axil of the injected leaf was completely permeated, and higher leaves were coloured, the intensity varying in different leaflets. This general distribution over the whole plant was in marked contrast to the localized distribution resulting from the injection of tobacco by the same method.

The arrangement of the strands supplying the leaves was ascertained by stripping off overlying tissues. There are three main strands running to the base of each leaf which occupy about three-quarters of the circumference of

the stem at that point. Each of these strands in passing down the main stem comes into contact with all of those of the leaves above and below it. This fact in part explains why a leaf-stalk injection permeates practically the whole of the soya-bean plant above the injection point.

(b) *With nutrients.* (i) *Nitrogen.* One of a number of plants which were shedding their leaves prematurely was injected with 0.2 per cent. urea. It



TEXT-FIG. 8. Distribution resulting from leaf-stalk injection of dye solution through a cut leaf stalk in the soya bean. Permeated areas are shaded according to the intensity of the colour.

(see Pl. III, Fig. 7, left) retained its leaves and put forth new ones, while all three untreated plants (one of which is shown on the right in Pl. III, Fig. 7) shed their leaves and put forth no new ones. The uninjected plants ultimately shrivelled and died, but the injected one remained green with a new set of leaves.

(ii) *Phosphorus.* Sodium dihydrogen phosphate (0.2 per cent.) was injected in two instalments at an interval of 1 week and the result is shown in Pl. III, Fig. 8. The left-hand plant was untreated and was typical of phosphorus-deficient plants, while the right-hand one was injected. The most striking difference was in the amount of new growth made in the injected plant. Leaves already expanded received little or no benefit.

(iii) *Potassium.* A solution containing 0.25 per cent. of a mixture of equal weights of potassium chloride and potassium sulphate was injected into one

plant. In the 3rd week the injected plant looked the worst of this series, owing to a severe infestation of red spider. There were a few spiders on the untreated plants, but the infestation was not comparable with that of the injected plant in spite of the plants being within a foot of each other.

On closer examination, however, it was found that the uninjected plants showed leaf scorch, characteristic of potassium shortage, while the potassium injected one was almost free from it.

(iv) *Boron*. The response to the leaf-stalk injection of boric acid is seen in Pl. III, Fig. 9. The plant on the right hand (injected) is free from mottled and chlorotic leaves, and the newer leaves arising are also quite normal. The older leaves are still mottled, showing that mature leaves do not respond.

SUMMARY

1. Injection methods for diagnosing mineral deficiencies have been worked out on tobacco and soya-bean plants grown under deficiency conditions.

2. In tobacco the leaf-stalk method was found the most useful for this purpose.

3. Injected dye solution in the second and third leaf above the injection point permeated only the side of the leaf nearer the injection point. Leaf 4 was not permeated at all by dye, but one-half of it was permeated by some of the nutrients.

4. The injection of solutions containing compounds of nitrogen, phosphorus, potassium, magnesium, and iron into tobacco leaf-stalks led, in 9 or 10 days, to an improvement in the colour of the proximal sides of leaves 2 and 3, and in addition in one side of leaf 4.

5. Growth increases were detected, both by a puckering of the surface and by actual measurement in the permeated compared with the unaffected halves of these leaves, following injections of potassium, magnesium, and iron.

6. Deficiencies of nitrogen, phosphorus, potassium, iron, and boron have been diagnosed in the soya bean by plant-injection methods.

7. The interveinal method was found to be the best in the soya bean for diagnostic purposes.

8. Iron deficiency was diagnosed in the soya bean by the interveinal method in 2 days.

9. Injection by the leaf-stalk method resulted in the permeation of nearly the whole soya-bean plant.

The author wishes to express his sincere thanks to the Director of East Malling Research Station for placing facilities at his disposal and to Dr. W. A. Roach, under whom this work was done, for much valued help.

He also wishes to thank Miss K. Cornford and Mr. L. N. Lavender for the photographic work and the latter for preparing the diagrams for publication.

LITERATURE CITED

- ROACH, W. A., 1938: Plant Injection for Diagnostic and Curative Purposes. *Imp. Bur. Hort. and Plant. Crops. Tech. Comm. No. 10*, 1938.
 — Plant Injection as a Physiological Method. *Ann. Bot., N.S.*, iii. 155-226.
 HILL, H., and ROACH, W. A., 1940: Injection for the Diagnosis of Mineral Deficiencies in the Tomato, the Potato and the Broad Bean. *Ibid.*, iv. 505-21.

EXPLANATION OF PLATE III

Illustrating Dr. Lal's paper on 'Plant-Injection Methods for the Diagnosis of Mineral Deficiencies in Tobacco and Soya Bean'.

Fig. 1. The effect of the injection of urea by the leaf-stalk method into a tobacco plant as seen in a single detached leaf. The permeated half is darker green than the unaffected half.

Fig. 2. Interveinal injection of urea in the soya bean. A darker area (greener in the original leaf) may be seen surrounding each incision.

Fig. 3. Interveinal injection of iron salt in the soya bean. The whole leaf was chlorotic except for the small areas (dark in the plate) which became perceptibly green the 2nd day after injection and a vivid healthy green on the 3rd day.

Fig. 4. Soya-bean leaf showing symptoms of boron deficiency. Small areas between the veins were devoid of chlorophyll.

Fig. 5. Interveinal injection of boric acid in the soya bean. A healthy area surrounded each incision in a leaf all other areas of which showed marked symptoms of iron deficiency.

Fig. 6. Leaflet-tip injection of soya bean with urea. The cut terminal leaflet was of an intense green and the proximal halves of the lateral leaflets were of a strikingly more healthy green colour than the untreated distal halves. This difference was much more striking in the living specimen than in the photograph.

Fig. 7. Plant on left injected with 0.2 per cent. solution of urea through a cut-leaf stalk. It retained its leaves and put forth new ones, whereas the uninjected plant on the right lost all its leaves.

Fig. 8. Right-hand plant injected with 0.2 per cent. solution of sodium dihydrogen phosphate through a cut-leaf stalk. Left-hand plant not injected.

Fig. 9. Right-hand plant injected with boric acid through a cut-leaf stalk. The left-hand one was not injected.



8



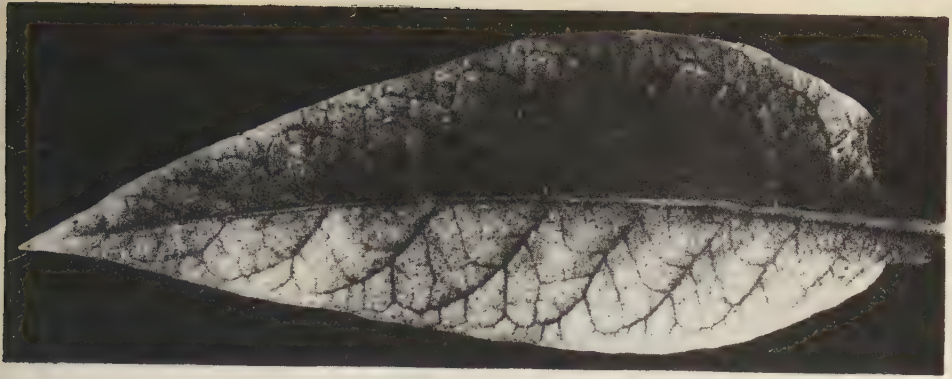
7



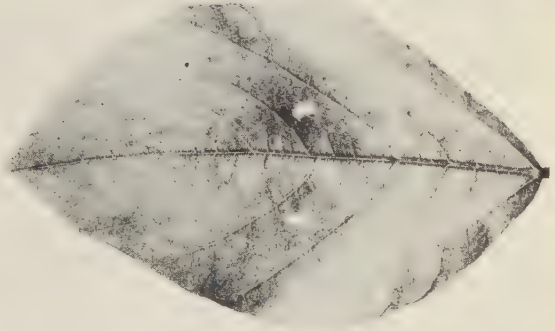
9

LAL - PLANT INJECTION.

Huth coll.



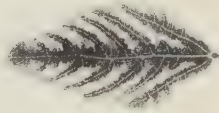
2



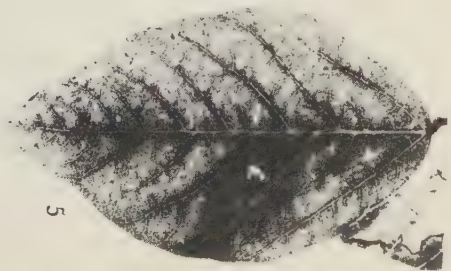
3



4



5



Studies on Foliar Hydration in the Cotton Plant

VI. A Gel Theory of Cell Water Relations¹

BY

E. PHILLIS

AND

T. G. MASON

(Cotton Research Station, Trinidad, B.W.I.)

With ten Figures in the Text

	PAGE
I. INTRODUCTION	297
II. THE DISTRIBUTION OF WATER IN THE CELL DURING SWELLING (<i>Experiment 1</i>) .	299
III. THE SIGNIFICANCE OF CONDUCTIVITY DETERMINATIONS (<i>Experiment 2</i>) .	300
IV. DIURNAL VARIATIONS (<i>Experiment 3</i>)	304
V. THE USE OF THE SALT-TRANSFORMATION	306
VI. THE EFFECT OF NON-ELECTROLYTES (<i>Experiment 4</i>)	309
VII. QUANTITATIVE RELATIONS BETWEEN SALT AND WATER	310
1. Variations due to the amount of salt, to cutting, and to age (<i>Experiment 5</i>) .	310
2. Variations due to other factors (<i>Experiments 5-19</i>)	313
VIII. SPECIFIC ELEMENT EFFECTS	314
IX. INERTNESS (<i>Experiment 21</i>)	317
X. CONDITIONING (<i>Experiment 19, Part 1</i>)	320
XI. CARRY-OVER (<i>Experiment 19, Part 2</i>)	322
XII. SWELLING ON NUTRIENT SOLUTIONS (<i>Experiment 22</i>)	328
XIII. DISCUSSION	330
XIV. SUMMARY	333
LITERATURE CITED	333

I. INTRODUCTION

IN a recent paper (Mason and Phillis, 1942 a) we showed that discs cut from cotton leaves might absorb considerable amounts of water when floated on certain salt solutions. This absorption of water was usually accompanied by an increase in the electrical conductivity of the expressed sap and always by an increase in the salt content (i.e. specific conductivity \times water) of the discs. Discs floated on solutions of non-electrolytes (e.g. sucrose) or on water showed no appreciable uptake of water. In a second paper (Phillis and Mason, 1943) we demonstrated that the remaining leaves on a partially defoliated plant ('pruning method') behaved in a similar manner to foliar discs when the roots were supplied with an electrolyte solution. To explain this effect of salts on the hydration of the cell we suggested that protoplasm

¹ Paper No. 34 from the Physiological Department of the Cotton Research Station Trinidad.

might behave in a manner analogous to gelatin in its iso-electric region (cf. Jordan Lloyd and Pleass, 1927).

We (Mason and Phillis, 1943) also investigated the relation between salt and water in the leaves of plants whose roots were exposed throughout development to nutrient solutions varying both in composition and in concentration. The plants were grown in a glass-house and every effort was made to avoid the *size effect* (cf. Mason and Phillis, 1942). Hydration was expressed on both the dry weight and protein bases and was found to be influenced by the level of *salt concentration* in the expressed sap.

The results obtained in the salt-swelling of foliar material could not be harmonized with the *classical* osmotic theory of cell-water relations. Furthermore, experiments on the expression of sap from cotton leaves (cf. Mason and Phillis, 1939; Phillis and Mason, 1941) led us to infer that much of the water in the cell (70–75 per cent.) is located in the protoplasm and not in the vacuole as had hitherto been generally assumed. Moreover, this protoplasmic water does not appear to be present as free water, but rather to be combined in some way as water of constitution. When protoplasm is killed this water is liberated, and is then obtained with other protoplasmic constituents as protoplasmic sap. Now protoplasm is generally recognized as a colloidal system (cf. Seifriz, 1936, 1942) and as such possesses *structure*. The water relations of colloidal systems such as gelatin gels have been explored. One hypothesis (cf. Jordan Lloyd, 1938) is that in such systems the water content is determined by the balance between osmotic forces in the sol in the interstitial spaces and the elastic forces in the framework. This hypothesis involves a two-phase system, but another view (cf. Von Buzagh, 1937) is that 'the question whether a swollen substance is a single-phase or a two-phase system is just as absurd as that whether disperse systems of independent particles are to be regarded as homogeneous or heterogeneous systems in the sense of the phase rule. . . . The common point about all kinds of swelling processes is that the liquid penetrates to the interior of the swelling substance, and is there linked by combining forces of some kind to the building units of the swelling substance, without entirely destroying the cohesion between the building units.'

On either view, it is clear that the cohesion between building units which constitutes the structure will influence the amount of water that can be taken in. Now in protoplasm it is believed that the structure is built out of protein molecules (cf. Seifriz, 1942). Salts are known to affect the hydration capacity of protein gels in their iso-electric region, and it has been inferred that they reduce in some way the cohesive forces between the molecules.

It might therefore be expected that the hydration capacity of protoplasm would be affected by its salt content, and this expectation accords with our observations on the relation between salt and foliar hydration. In this paper we present further evidence for the hypothesis that the hydration capacity of the foliar tissues of the cotton plant is largely controlled by protoplasmic imbibition, and that this in its turn is controlled by the salt content.

II. THE DISTRIBUTION OF WATER IN THE CELL DURING SWELLING (Experiment 1)

A. Introduction.

In our previous hydration papers we have dealt only with the total water content. Evidence from sap-expression experiments as well as from direct microscopic observation led us to infer (cf. Mason and Phillis, 1939; Phillis and Mason, 1941) that in the normal mature cotton leaf the major part of the water (70-75 per cent.) is located in the cytoplasm. Our claim to have separated the vacuolar from the protoplasmic sap has received support from the work of Bennet-Clark and Bexon (1939). Before we can discuss the mechanism of salt-swelling in foliar material it is essential to know whether there is a differential response between cytoplasm and vacuole. Hoagland and Broyer (1942) record that when *Nitella* cells accumulate radioactive rubidium or bromine, the solute first of all accumulates in the cytoplasm to a higher concentration than in the external medium, and that there is a continued movement of solute from the cytoplasm into the vacuole, so that in the course of time the concentration in the vacuole exceeds that in the cytoplasm. It might be expected, with the massive salt accumulation that we have observed in discs, that much of the solute would be located in the vacuole, and this might lead to much of the water taken up for osmotic reasons also being located in the vacuole.

Swollen discs are very difficult to press in such a manner that a clear separation of vacuolar and cytoplasmic saps is obtained. Not only is it much more difficult to make from discs than from leaf strips a wad which will stand pressure without slipping, but salt-swollen discs are much more brittle than normal material and are much more difficult to pack since swelling is normally accompanied by buckling. We have therefore had recourse to the 'pruning method' to obtain salt-swollen material which could be satisfactorily used for the estimation of the distribution of water between vacuole and cytoplasm.

B. Procedure.

Plants were grown in sand-culture supplied with a full nutrient solution. When they were 9 weeks old the main-axis leaves on nodes 6, 7, 8, and 9 from the apex were marked with wool. The remaining leaves (about 75 per cent. of the total) and the fruiting-branches were then removed. Next the sand was leached with tap-water. The plants were then graded and divided into 4 groups as follows:

Group 1. Leaves collected immediately (Initial collection).

" 2. Full nutrient solution replaced by water.

" 3. " " " " M/50 CaCl_2 .

" 4. " " " unchanged.

The leaves from groups 2, 3, and 4 were collected 18 days after the Initial collection. Each group contained two samples of twelve plants so that there

were 48 leaves per sample. Vacuolar and cytoplasmic saps were expressed in the usual way. On these saps and that expressed from the whole leaf we determined the specific conductivity at 0° C. and the concentrations of chlorine and potassium. Three estimates of the distribution of water between vacuole and cytoplasm are thus available.

C. Results.

The results are shown in Table I. On the left are shown the changes in water content. The mean initial weight of water has been made 100. It will be seen that the Water group increased its water content by 47.7 per cent. It seems possible that leaching of the sand was not complete for the conductivity of the sap of the Water group has increased. The Calcium Chloride group increased its water content by 69.4 per cent. and the Full Nutrient group by 143.8 per cent. The concentrations of electrolytes (i.e. specific conductivities) of chlorine and of potassium in the vacuole, the protoplasm, and in the whole leaf are shown in the body of the table.

From these concentrations we have calculated the percentages of the total water located in the vacuole. The means for each group are shown on the extreme right of the table and the means for each solute at the bottom. It will be seen that calculation from specific conductivity gives the lowest value (24.2 per cent.) and from chlorine the highest (26.2 per cent.). The difference is small and not significant.

We come now to the main object of the experiment. The initial percentage of water in the vacuole was 25.4. Though there were marked increases in the water content and in concentration in all groups, yet the distribution of water in the cell remained practically unchanged. In the Full Nutrient group the percentage of water in the vacuole dropped to 24.7, while in the Calcium Chloride group it rose slightly to 25.5, but these changes are not significant.

D. Conclusion.

Most of the water taken in during swelling is located in the protoplasm. This follows from the fact that approximately 75 per cent. of the total water is initially present in the protoplasm and the distribution of water between protoplasm and vacuole does not change during swelling.

III. THE SIGNIFICANCE OF CONDUCTIVITY DETERMINATIONS

(Experiment 2)

A. Introduction.

All the subsequent experiments described in this paper are concerned with discs floated on solutions. We have used electrical conductivity of the expressed sap as a measure of electrolyte concentration in the sap, and have calculated salt from the specific conductivity of the sap \times the weight of water per 1,000 discs. Though we have used conductivity determinations in our earlier papers, it seems desirable to say something further about their significance.

TABLE I

Water Content (expressed as percentages of mean initial value), Specific Conductivity ($C \times 10^3$), and Concentrations (mg. per 100 gm. water) of Chlorine and Potassium in Saps expressed from Vacuole, Protoplasm, and Whole Cell, together with Percentages of Water in Vacuole as calculated from these Values

Group.	Water content. { a b }	Mean.	Sp. conductivity			Chlorine			Potassium			Percentage of water in vacuole calculated from		
			Vacuole	Protoplasm	Whole cell	Vacuole	Protoplasm	Whole cell	Vacuole	Protoplasm	Whole cell	Sp. cond.	Chlorine	Potassium
Initial	{ a 99.0 b 101.0 }	100.0	{ 1.6 1.6 }	{ 21.1 18.9 }	{ 15.6 16.7 }	{ 41 47 }	{ 562 598 }	{ 426 450 }	{ 49 75 }	{ 797 851 }	{ 626 663 }	{ 28.1 24.6 }	{ 26.0 26.9 }	{ 22.8 24.2 }
Water	{ a 147.6 b 147.7 }	147.7	{ 2.3 2.5 }	{ 24.2 24.7 }	{ 19.4 19.4 }	{ 59 50 }	{ 726 710 }	{ 550 526 }	{ 57 65 }	{ 956 877 }	{ 719 679 }	{ 22.1 23.9 }	{ 26.5 27.8 }	{ 26.3 24.3 }
Calcium Chloride	{ a 172.5 b 166.2 }	169.4	{ 4.3 4.5 }	{ 27.4 28.8 }	{ 21.4 24.1 }	{ 129 173 }	{ 1,190 1,342 }	{ 857 1,010 }	{ 77 103 }	{ 707 694 }	{ 547 563 }	{ 25.9 19.3 }	{ 31.4 28.4 }	{ 25.5 22.2 }
Full Nutrient	{ a 250.5 b 237.0 }	243.8	{ 5.0 5.7 }	{ 25.4 25.5 }	{ 20.6 20.5 }	{ 95 113 }	{ 473 467 }	{ 402 382 }	{ 130 138 }	{ 990 972 }	{ 755 737 }	{ 23.8 25.9 }	{ 18.8 24.0 }	{ 27.4 28.2 }
												Sig. Diff. ($P = 0.05$)		
												24.2	26.2	25.1
												Sig. Diff. ($P = 0.05$)		
												3.0		

Sig. Diff. ($P = 0.05$) 3.7

Mean 24.2 26.2 25.1

Sig. Diff. ($P = 0.05$)

Conductivity determinations were first used by Dixon and Atkins (cf. Atkins, 1916) as a rapid and convenient method of estimating the concentration of electrolytes in saps. Their use has been adversely commented on by Haynes (1919), Mason (1919), and rather recently by Greathouse (1938). The latter concluded that 'it is almost impossible to translate conductivity values of mixed salts or plant saps into concentration values'. Our problem is, however, somewhat simplified by the fact that in most of our experiments we are dealing with the accumulation of a single salt (viz. calcium chloride) in the cell. The question we have to answer is whether changes in salt concentration in sap due to the addition of a single salt can be determined from conductivity measurements.

B. Procedure.

Leaf discs were floated on two solutions of calcium chloride (viz. M/30 and M/75). In addition to the Initial, there were collections after 2, 5, 8, and 12 days. Samples were taken for the determination of water, of conductivity of expressed sap, and of chlorine. Chlorine was determined on the dried material (cf. Mason and Phillis, 1936). Immediately on removal from the calcium chloride solutions *the discs were well washed in several changes of water and then dried between blotting-paper*. Conductivity determinations were made at 0° C. on sap diluted 10 times. The results are corrected for this dilution.

C. Results.

The weights of chlorine (determined on dried material) and of salt (calculated from conductivity \times water) per 1,000 discs are plotted against each other in Fig. 1. It will be seen that they are very highly correlated ($r = +0.995$) in a linear manner over most of the range covered. The magnitude of the correlation shows that *chlorine* can be estimated from *salt* with considerable precision (cf. Treloar, 1936). It is immaterial therefore whether chlorine be determined on the dried material or by conductivity measurements on the expressed sap.

It is possible to calculate from the increase in chlorine and in water what the conductivity increase should be, assuming that the change in chlorine is entirely due to calcium chloride uptake. When such a calculation is made for the last collection on the M/30 calcium chloride solution, a final conductivity value of 31.89×10^{-3} mhos is indicated. The value actually found was 28.4×10^{-3} mhos. The difference is in the reverse direction to what would be expected if bound water (cf. Mason and Phillis, 1936) was present.

D. Conclusion.

Conductivity measurements on expressed sap and weight of water in the sample can be used satisfactorily to estimate the changes in the amount of calcium chloride (i.e. salt) in the sample.

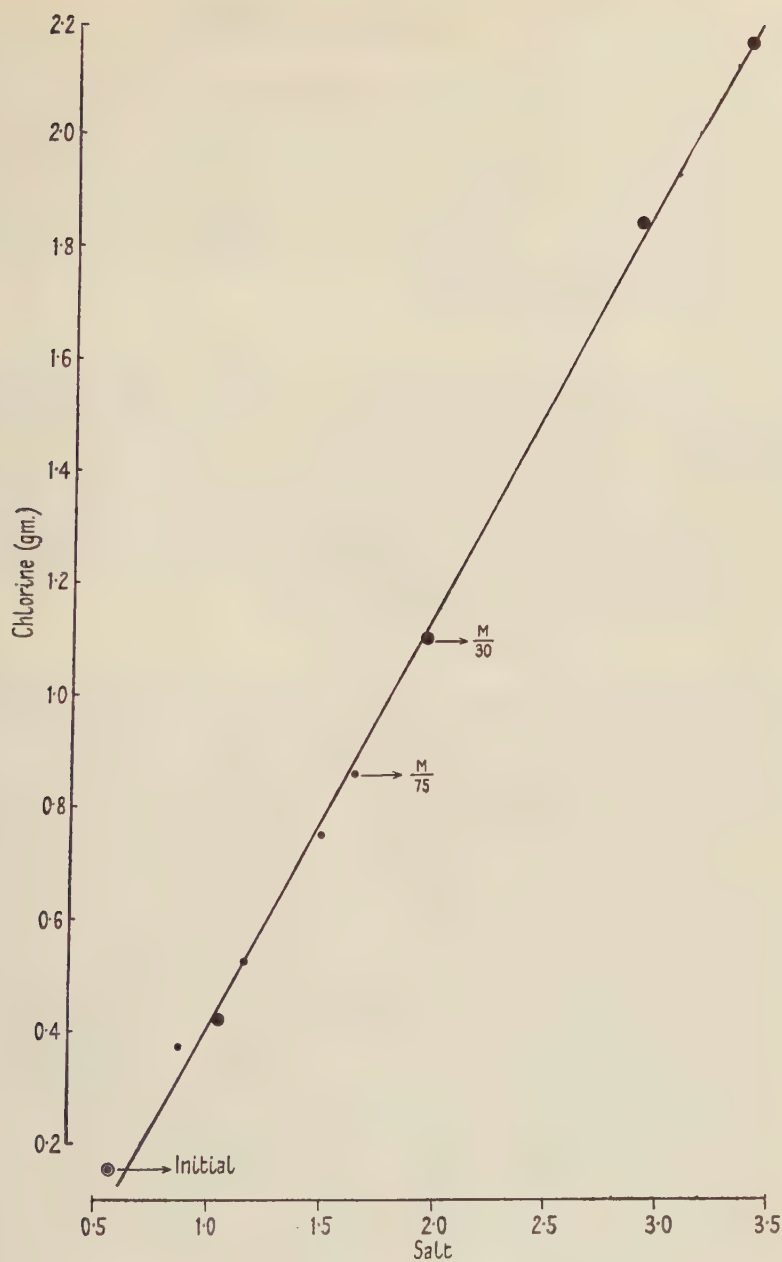


FIG. 1. Relation between the amounts of chlorine and of salt per 1,000 discs

IV. DIURNAL VARIATIONS (*Experiment 3*)A. *Introduction.*

The normal disc technique in which discs are floated on solutions in trays situated in a greenhouse involves exposure of the discs to alternating periods of light and darkness. It was therefore deemed desirable to ascertain the effect of this alternation on water and salt uptake. There is of course nothing to hinder the use of the 'disc technique' under conditions of constant light, but it is more convenient to avoid using artificial lighting with its problems of heat disposal, insect attraction, &c.

B. *Procedure.*

Discs were floated both on water and on M/50 calcium chloride. Collections were made daily at 3.00 p.m. and 8.00 a.m. for 4 days. The trays holding the discs were covered with wooden screens at 3.00 p.m. and the screens removed at 8.00 a.m. At each collection 2 samples were taken. New blotting-paper was used throughout for drying the discs in order to obtain uniform drying. All discs were washed in several changes of water and remained wet for about 20 minutes before drying was completed. Calcium was determined in the expressed sap (cf. Phillis and Mason, 1940).

C. *Results.*

The results, which are expressed as percentages of the values at the Initial collection, are shown in Fig. 2. Water, salt, and calcium are expressed on the sample basis; salt is, as usual, calculated from specific conductivity \times weight of water per sample. Conductivity was determined on the expressed sap.

We will consider first the changes in water content. In the water treatment there was no appreciable trend during the course of the experiment, while on calcium chloride water content increased by more than 75 per cent. In the water treatment there were well-defined diurnal variations. These variations ranged from 2 to 5 per cent. and were only slightly less than the diurnal variations found in leaves on plants growing in the open (cf. Phillis and Mason, 1942). As the discs were thoroughly wetted with water for at least 20 minutes on collection, a saturation deficit is unlikely and some change in protoplasmic hydration capacity due to light is possibly indicated.¹ Diurnal changes in moisture content of the discs floated on calcium chloride are less regular. We have attempted to allow for the diurnal moisture changes of the calcium chloride discs. The following procedure was used: the evening values were raised by the percentage drop shown by the evening values of the water treatment below the preceding morning values. The corrected water content is shown on the graph. It will be seen that the corrected line is nearly straight.

It would thus appear, when allowance is made for the diurnal changes in

¹ It might also be due to differential drying between the morning and afternoon collections, but we think that the possibility of all the observed difference being due to this cause is remote.

environment which were responsible for the diurnal changes in the water content of the discs floated on water, that water entered the calcium chloride

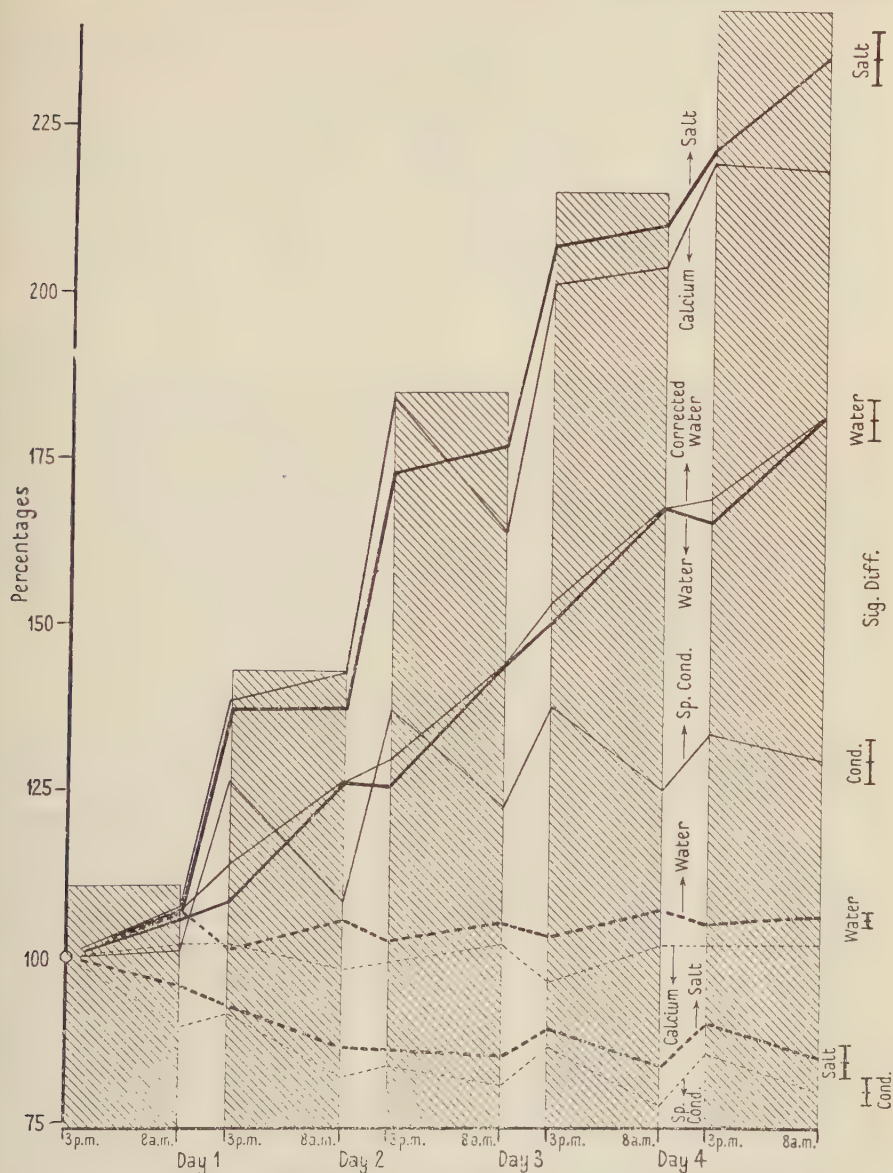


FIG. 2. Diurnal changes in water, salt, specific conductivity, and calcium in discs floated on water (broken lines) and on calcium chloride solution (continuous lines). Results are expressed as percentages of initial values. Dark periods are shaded.

discs at an approximately constant rate by day and by night. Now salt entered much more rapidly by day; in fact, the salt uptake during the night was

negligible. As calcium and salt showed very much the same changes, it seems clear that salt is a satisfactory indicator of the changes in the rate at which calcium chloride entered the discs. It is evident that the effect of salt on water uptake persists after salt uptake has ceased. We have termed this continued uptake of water in the absence of salt uptake *carry-over*, and the phenomenon is more fully discussed in a later section.

It will be noticed that the maximum conductivity was reached in 48 hours, after which, apart from diurnal variations, there was no further change.¹ Thus, from 48 to 114 hours water and salt were entering the discs at about the same rate, and conductivity did not change. It seems clear that in this experiment salt and not conductivity must have been responsible for the uptake of water after 48 hours.

D. Conclusion.

In discs floating on water there are well-marked diurnal variations in water content. Discs floated on salt solutions take up salt only during periods of illumination, whereas water intake in such discs appears to be a continuous process. In experiments running for several days it is therefore desirable to collect discs at a definite time each day. We have collected as soon after dawn as conveniently possible. It would also appear that water may continue to enter after salt uptake has ceased. The effect of salt persists for some time and this we have termed *carry-over*. It seems that the amount of salt rather than its concentration in terms of water is the determining factor.

V. THE USE OF THE SALT-TRANSFORMATION

Richards (1944) says: 'When it is desired to investigate the effect of salt content on water content, therefore, independent methods of estimating both should be sought.' 'The "salt" transformation', he points out, 'may improve the simple correlation coefficient in two ways: (1) by straightening the regression line and (2) in a purely algebraic manner by introducing a factor common to both variates.' By 'salt transformation' he of course refers to our procedure of estimating salt from the product of conductivity and water.

However true this may be, the fact is that salt (i.e. sap-soluble salt) cannot yet be estimated independently of water. To do so the determination would have to be carried out on the dried material or on the extract obtained with hot water, and these processes lead to changes in the adsorbed fractions. All the cations (calcium, potassium, and magnesium) thus far investigated (cf. Phillis and Mason, 1940) contain large and highly variable adsorbed fractions which are released on heating. Chlorine alone can be determined on the dried material or by extraction, for the whole of it seems to be sap-soluble (cf. Mason and Phillis, 1936).

He also states 'by substituting "salt content" (i.e. conductivity \times water

¹ In this respect the experiment was exceptional, for conductivity normally increases for a longer period.

content) for conductivity a substantial correlation with water content must necessarily appear'. This is, however, only true if conductivity and water are positively correlated. Further, the 'salt transformation' may lower the correlation with water when conductivity and water are positively and perfectly correlated. He is right, however, when he points out that any improvement that does emerge as a result of the 'salt transformation' is *spurious*. This really is the base of his criticism. We will examine to what extent the use of the 'salt transformation' may have misled us. The fact that sap-soluble salt cannot be estimated directly does not affect the issue.

His statement that 'the magnitude of the correlation coefficient obtained is the sole criterion used by Mason and Phillis in discriminating between the effects of conductivity and "salt"', is not correct. It is not correct because not only was it not the 'sole criterion' but in no single instance was it even a contributory criterion.

Thus, in experiment 2 of our paper (Mason and Phillis, 1942*a*) using the 'Disc-culture Method', discs were floated on a full nutrient solution and the changes in conductivity and water followed. The results are shown graphically in Fig. 2 of the paper. It was found that water¹ continued to increase for the 13 days the experiment lasted, while conductivity showed very little change after the first day. Salt content was estimated and was also shown on the graph. In commenting on the results we said 'changes in water content and conductivity are quite different'. Also, 'the changes in water content were rather similar to those in salt content, but this of course is due to the fact that the salt content changes are due almost wholly to changes in water content, the changes in conductivity being relatively small. Protein changes were not determined and there is the possibility that water uptake was due to an increase in the protein content of the cell.' It is noteworthy that the data were not submitted to statistical analysis and correlation coefficients were not even calculated, much less compared.

In the third experiment of the same paper the results were also shown graphically and judged by inspection. The correlation coefficient between conductivity and water was not even recorded, much less was it compared with the salt correlation as a criterion in discriminating between the effects of conductivity and salt.

In this experiment the discs were floated on water and on solutions of calcium chloride of different concentrations. The experiment continued for 15 days. The discs on water showed a small loss in water, and those on calcium chloride increases which were proportional to the concentration of calcium chloride in the external solution. In this experiment there were no complications due to the use of a full nutrient solution, so that the uptake of water must have been in some way due to the uptake of calcium chloride. The question at issue, therefore, was to decide whether the uptake of water was due to changes in the *concentration* of calcium chloride or to the *amount* of calcium chloride. As the water changes were unlike those of conductivity

¹ Water was expressed on the sample basis and not, as Richards states, per 100 gm. dry weight.

in *pattern* and in one instance in direction, it was concluded that the uptake of water was due to the amount of salt taken up rather than to the changes in concentration of salt.

In this experiment the discs were re-cut at each collection in order to expose a fresh surface to the salt solution. Later we found that in the presence of salt re-cutting the discs might have a marked effect on water uptake. In our work, using the 'pruning method' (cf. Phillis and Mason, 1943), we were also led to suggest that the response to salt might be influenced by some 'breakdown in protoplasmic structure'. We were therefore led to state: 'A more critical examination of the results . . . has inclined us to the view that the evidence at present available does not enable us to decide whether the conductivity of the sap or the amount of salt is the more important.'

In our third paper (Mason and Phillis, 1943) we grew plants in nutrient solutions varying in composition and concentration. Our object was to find out whether salt was *in any way* responsible for the changes in hydration observed in the leaves, for non-salt factors might have been at work in such experiments. Our object was not, as in our previous experiments, to find out whether salt affected water content as a concentration or as an amount, and consequently we only considered changes in 'conductivity' except in one experiment in which we used 'salt' to compare the effects on hydration of different elements in the nutrient solution. No comparison was made of the strength of the correlations between water and conductivity and between water and salt. Moreover, no conclusions were drawn from the inspection of graphs as to whether salt acted as a concentration or as an amount in influencing water content.

This was the position (viz. no decision as to concentration *versus* amount) when the work reported in the present paper was begun. In the experiment reported in the previous section we noted that the conductivity changes and the water changes were not related. The discs had not been cut, and as the experiment ran for only 4 days there is no suggestion of protoplasmic breakdown. The uptake of water must consequently have been due in some way to the salt taken up, for discs on water did not increase their water content. We were thus left with the choice of concentration or amount. As conductivity showed no appreciable change we were left with salt, and concluded consequently that amount and not concentration of salt was instrumental in drawing water into the cell.

Had we considered the data statistically and taken no account of the background of the experiment, we might have concluded that salt was not acting as a concentration since conductivity did not change after 48 hours while water did, and that there was no evidence that it was acting as an amount because the correlation coefficient between water and salt is wholly spurious. The fact is that when water uptake is accompanied by no change in concentration it is because water and salt are entering at the same relative rate, and if this water uptake is not due to a third factor (e.g. new protoplasm) there is a *prima-facie* case that the amount of salt is affecting hydration.

In the diurnal experiment considered in the previous section we pointed out that water continued to enter during the night after salt absorption had ceased. We termed the continued uptake of water *carry-over*. It might be suggested that some similar phenomenon was at work with respect to conductivity, and that the continued uptake of water after 48 hours was due to a *carry-over* effect of the rise in conductivity during the first 48 hours. This possibility does not seem feasible. We have found that the rate of water uptake due to *carry-over* rapidly falls off in time, reaching zero in about 60 hours in normal material. But water uptake proceeded at a more or less steady rate throughout the whole of this diurnal experiment.

VI. THE EFFECT OF NON-ELECTROLYTES (*Experiment 4*)

A. Introduction.

We have alluded in previous papers to the apparent ineffectiveness of non-electrolytes in promoting swelling. Thus discs floated on sucrose solution did not swell. Again, discs floating on water showed an increase in time in the freezing-point depression of the sap with simultaneous declines in conductivity and water content, while in another experiment hydration in leaves was found to be significantly correlated with conductivity but the correlation with freezing-point depression was small and not significant; when, however, the latter was corrected for the concentration of sugars in the sap, the correlation was raised to the level of significance.

Now none of these observations can be regarded as conclusive evidence that non-electrolytes cannot promote swelling. The absence of swelling on sucrose solutions might, for instance, be due to failure to build up a sufficient sugar concentration in the discs. We therefore planned an experiment in which discs were floated on calcium chloride and on sucrose solution of such strength that freezing-point depression increased at much the same rate on the two solutions.

B. Procedure.

Discs were floated on (1) water, (2) M/100 calcium chloride, (3) 6 per cent. sucrose solutions. Collections were made early next morning (day 1) and again 3 days later (day 4). At each collection water content was determined, while conductivity, freezing-point depression, and total sugar concentration were estimated on expressed sap. The experiment was not continued beyond 4 days owing to difficulty in preventing increasing contamination of the sucrose solution.

C. Results.

The results are shown in Table II. It will be seen that by day 4 the freezing-point depression of the sucrose discs had increased by 0.20°C . over the water discs, while the increase for the calcium chloride discs was 0.18°C . But, whereas the calcium chloride discs showed a fully significant *gain* over

the water discs of 3.19 gm. water, the sucrose discs showed a *loss*, which was not significant, of 1.67 gm. water.

If instead of using the water results as a basis, trends in time are compared, it will be seen that between days 1 and 4 the discs on sucrose showed an increase in freezing-point depression of 0.08°C. , those on calcium chloride an increase of 0.13°C. , and the water discs an increase of 0.04°C. , all increases

TABLE II

Water and Salt Content (gm. per 1,000 discs) and Concentrations of Total Sugar and Total Solutes (Freezing-point Depression) in Sap expressed from Discs floated on Water, Sucrose, and Calcium Chloride Solutions

Treatment.	Day.	Water content.	Salt content.	Total sugar (gm. per 100 gm. water).	Depression of freezing-point.
Water . . .	1	46.28	0.455	1.01	0.72°C.
	4	46.88	0.442	1.51	0.76°C.
Sucrose . . .	1	46.15	0.457	2.73	0.88°C.
	4	45.21	0.429	3.06	0.96°C.
Calcium chloride . .	1	45.49	0.520	0.93	0.81°C.
	4	50.07	0.740	0.96	0.94°C.
Sig. diff. ($P = 0.05$)	—	2.26	0.049	0.19	0.02°C.

being significant. Only the calcium chloride discs showed any significant increase in water content during this period. It seems clear that for comparable increases in total solute concentration as indicated by freezing-point depression, sucrose has been quite ineffective in inducing water uptake, whereas calcium chloride has brought about a significant increase in water. Sucrose did not apparently alter the salt content of the discs, while calcium chloride solution did increase it significantly. Electrolytes rather than total solutes are indicated as the cause of swelling.

On day 4 the discs on sucrose solution contained 1.55 gm. sugar per 100 gm. water in excess of that in the water discs. Even assuming this to be present as hexose rather than sucrose, this would only account for a difference in depression of 0.16°C. , whereas the actual difference was 0.20°C. It would appear that some sucrose must have been converted into non-sugar compounds.

D. Conclusion.

Sugars and products derived from sugars in the leaf appear to be ineffective in bringing about water absorption.

VII. QUANTITATIVE RELATIONS BETWEEN SALT AND WATER

1. Variations due to the amount of salt, to cutting, and to age (Experiment 5)

A. Introduction.

In our previous paper describing our 'disc' technique we showed in one experiment with calcium chloride that the relation between salt and water

was linear over a considerable range. The discs were, however, cut between collections, and the possibility arises that cutting affected the salt/water relation. In the present experiment we have used Normal or Uncut discs and have compared their behaviour with that of Cut discs. We have also compared the behaviour of discs punched from Old and Young leaves. In a previous section of the present paper we have shown, when discs are floated on calcium chloride solutions, that our salt estimate calculated from conductivity \times water is a satisfactory measure of changes in the amount of calcium chloride in the sample.

B. Procedure.

Discs were punched from leaves of two different ages. The Young leaves were taken from the 5th node and the Old from the 20th node of the main axis. The discs from the Old leaves did not float well and had to be discarded after the 4th day. There was no such difficulty with the discs from the Young leaves and collections were made on days 0, 1, 4, 7, 10, 14, and 21. The discs were floated on water and three calcium chloride solutions (viz. M/30, M/60, M/120). Immediately after the collection on the 7th day, half of the remaining discs were divided diametrically and the two halves refloated on their appropriate solutions.

C. Results.

The relation between salt and water is shown in Fig. 3. The values for the Old discs are shown by squares and those for the Young by circles in the figure. It will be seen that a linear relation holds over most of the range. Only the points for the high salt levels in the Normal discs and the highest salt level in the Cut series depart from this linear relation. We have calculated the correlation coefficient between water and salt for water increases up to 100 per cent. of the initial value. The coefficient amounts to $+0.992$. We shall refer to the regression coefficient of water on salt as the *water equivalent*.

The values for Old and Young discs lie near to the same regression line. Increasing hydration as leaves age on the plant would therefore be due to an essentially similar cause to swelling on calcium chloride solution. This seems a reasonable inference, since we know that both calcium and chlorine accumulate to a marked extent as leaves age (cf. Phillis and Mason, 1940, for calcium data).

When the regression line for water on salt is extended beyond the 100 per cent. limit, it will be noted that the extension lies close to most of the Cut values, but that the Normal values lie below it. Thus cutting, which has had little effect on the *water equivalent* at the lower salt levels, has, at the higher levels, temporarily restored it to its original value.

It will be seen that *all* the points for the Young discs floated on the solution of lowest concentration lie on the regression line. It may therefore be concluded that the divergences shown by the high salt values for the Normal discs cannot be attributed to time, but must rather be associated in some way

with the high salt content. This might suggest that the salt effect on water was reaching a saturation value were it not for the fact that cutting has at these levels resulted in further appreciable increases in water content. It may be that the salt effect on water diminished at the high salt levels of the

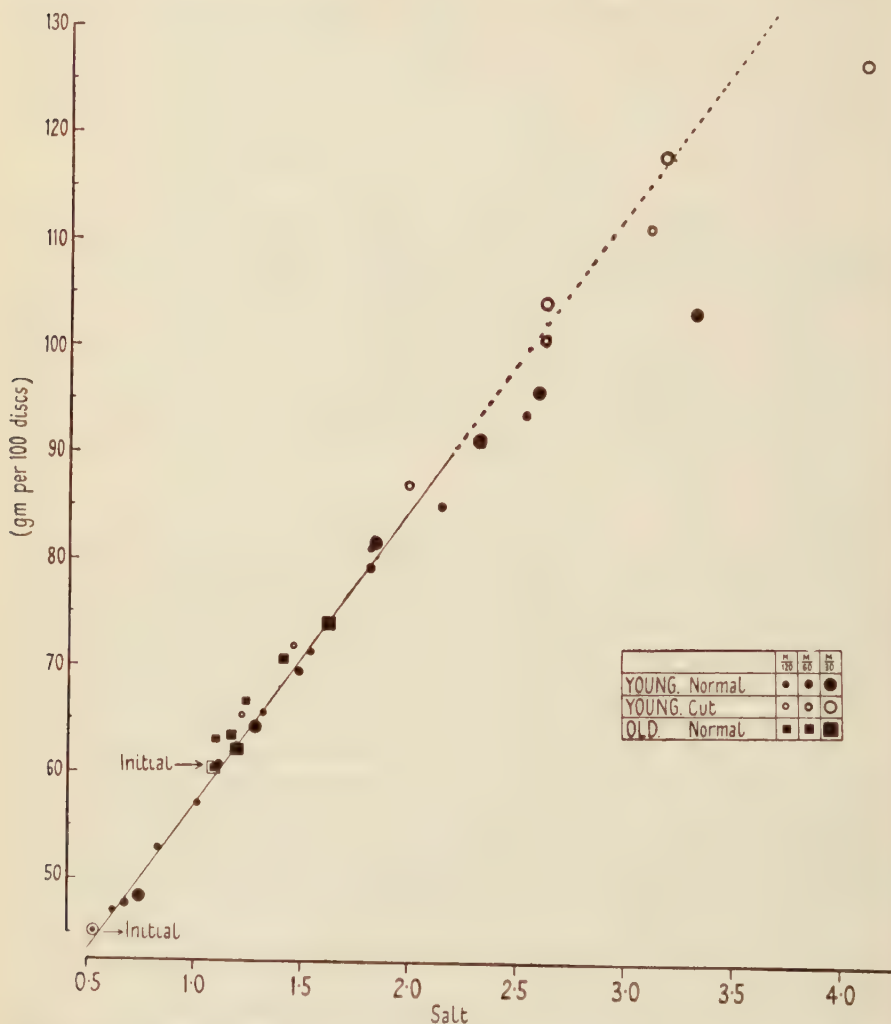


FIG. 3. The relation between salt and water in Young (normal and cut) and Old discs floated on calcium chloride solutions, with the calculated regression line of water on salt for water increases up to 100 per cent. of the initial water content.

Normal series because a limit to cell-wall extensibility was being approached, and that cutting, perhaps through the agency of a wound hormone, restored extensibility. It is also possible that the extensibility of the framework of the protoplasm may have to be reckoned with.

The existence of a linear correlation over such a wide range might suggest that a *definite amount of salt is causally associated with a definite amount of water*. To attribute it to the *salt-transformation* is to overlook the fact that our estimate of salt has the same value as a direct determination.

D. Conclusion.

It is shown that a linear relation holds between salt and water over a considerable range. At high salt values there is a diminution in the *water equivalent*. This change is delayed by cutting the discs. It is suggested that the cell wall or the framework of the protoplasm or both become less extensible at very high salt values and that cutting restores extensibility. The results for Old discs cover only a very limited range, but over this range there is no marked difference in the *water equivalents* of Young and Old discs. It is pointed out that increasing hydration as leaves age on the plant, and increasing hydration in discs floated on calcium chloride solutions, appear to be due to the same cause.

2. Variations due to other factors (Experiments 5-19)

A. Introduction.

The very high linear correlation in experiment 5 between salt and water for water increases up to 100 per cent. of the initial value (see Fig. 3) led us to investigate the relation in other experiments. In all, the data for 15 experiments with cotton-leaf discs on calcium chloride solutions were available for this purpose. These experiments covered a wide range of conditions and we have attempted to account for variation in the *water equivalent* in terms of variations in experimental conditions.

B. Procedure.

These experiments all followed the same general pattern in that discs were floated on calcium chloride solutions. There were wide variations in the concentrations used from experiment to experiment. In some experiments light intensity was varied, in others length of exposure to light. In some experiments the discs were floated directly on salt solutions, in others they were floated on water for a day before transfer to salt solutions. The leaves from which the discs were cut were highly variable. In all experiments discs from young leaves (about node 7) were used. The initial weight of water varied from 39.16 gm. (expt. 11) to 53.40 gm. (expt. 16) per 1,000 discs.

C. Results.

The results of these experiments are summarized in Table III. There was a tendency in some, but by no means all, experiments for the water changes to lag behind the salt changes at high levels as in expt. 5. For this reason, and since there was *no departure from the linear relation below 100 per cent. water increase*, the correlation and regression coefficients have been calculated only for this range.

Within this limit, water and salt were very highly correlated in each experiment. In any one experiment, then, the amount of water taken up was predominantly determined by the amount of salt absorbed, variations in the conditions under which this salt was absorbed having little influence.

The regression coefficients of water on salt, which we have termed the

TABLE III

Initial Weights of Water per Sample of 1,000 Discs. Correlation and Regression Coefficients of Water on Salt for fifteen Experiments in which Discs were floated on Calcium Chloride Solutions

No. of expt.	Initial water (gm).	No. of cases.	Correlation coefficient.	Regression coefficient.
5	45.18	19	+0.992	27.6
6	43.48	7	+0.987	24.9
7	40.94	7	+0.993	27.3
8	40.39	13	+0.984	27.7
9	44.61	19	+0.993	25.2
10	43.65	15	+0.994	29.7
11	39.16	9	+0.985	33.7
12	43.80	59	+0.986	29.4
13	41.46	7	+0.981	28.9
14	47.61	19	+0.956	24.1
15	41.09	13	+0.989	30.5
16	53.40	13	+0.986	23.0
17	44.20	10	+0.978	29.9
18	48.62	6	+0.986	24.0
19	44.21	7	+0.992	31.2

water equivalents, varied unduly, viz. from 23.0 (expt. 16) to 33.7 (expt. 11). Inspection of Table III suggested that the variation in the *water equivalents* was associated with variations in the initial water contents. The correlation coefficient between these is negative and fully significant ($r = -0.745$). Since standard-size cutters ($\frac{3}{8}$ in.) were used throughout, the initial water content may be taken as a measure of the weight of water per unit area. The meaning of this negative correlation between the *water equivalent* and water per unit area is not clear (see Discussion).

D. Conclusion.

Salt and water were very highly correlated throughout a series of experiments in which discs were floated on calcium chloride solutions. There was considerable variation in the *water equivalents* in different experiments. These variations were negatively correlated with variations in the initial weight of water per unit area.

VIII. SPECIFIC ELEMENT EFFECTS

A. Introduction.

There seems to be very little information on the relation between specific ions and hydration in plants. Salt (sodium chloride) succulence (cf. Meyer, 1931)

and nitrogen succulence (cf. Pearsall and Ewing, 1929) have both received attention, but there seems to be no agreement as to the nature of the mechanism at work. Richards and Shih (1940) concluded from their experiments that most of the variation in the water content (expressed in terms of dry weight) of barley leaves could be accounted for by variations in sodium, calcium, and phosphorus, with sodium playing the predominant part. Mason and Phillis (1943), on the other hand, could find no evidence of differential effects between calcium and potassium, but did find evidence of a specific effect due to phosphorus (cf. Richards, 1944).

B. *Comparison of the Effects of Sulphates and Chlorides and of Sodium and Potassium (Experiment 20).*

1. *Procedure.* Discs were floated on solutions of potassium chloride, sodium chloride, and potassium sulphate. There were three concentrations of each, viz. E/25, E/50, and E/100. The solutions were made up from pure anhydrous salts by direct weighing. The experiment covered a period of 19 days during which there were 5 collections.

2. *Results.* The results are shown in Fig. 4. The comparisons between potassium chloride and potassium sulphate and between potassium chloride and sodium chloride are shown separately on the graph. The regression of water on salt (i.e. the *water equivalent*) and the actual values from which they were calculated are shown. The values for the three different concentrations of the external solutions are marked distinctively (see graph). The regression lines calculated from the actual values are shown by continuous lines. It will be seen that there are well-marked differences between treatments. Thus sodium has a higher *water equivalent* than potassium. It will also be seen that chlorides have a higher *water equivalent* than sulphates.

Actual determinations over the range of concentrations covered in the sap showed that the equivalent conductivities of potassium chloride, potassium sulphate, and sodium chloride were in the proportion of 1:0.88:0.835. The increases in *salt* due to potassium sulphate and sodium chloride were, therefore, underestimates when compared with increases due to potassium chloride. We have adjusted the sulphate increases by raising them by the ratio 100:88, and the sodium chloride increases by the ratio 100:83.5. This adjustment of the sulphate and sodium chloride values to the potassium chloride level enables the *water equivalents* for the three salts to be directly compared. The regression lines for potassium sulphate and sodium chloride, calculated from the adjusted values, are shown in Fig. 4 by broken lines. Thus sodium and potassium (as chlorides) appear to be equally effective in increasing hydration, while potassium sulphate is not nearly so effective as the chlorides.

An objection might be raised to this conclusion on the grounds that while we have demonstrated the validity of conductivity measurements for calcium chloride, we have not done so for the three salts employed in the present experiment. In addition to conductivities, however, we also determined

freezing-point depressions, and have calculated 'total solutes' by multiplying together freezing-point depression in degrees Centigrade and water content in grammes per sample of 1,000 discs. We then calculated the regression coefficients of total solute on water. The values so obtained are shown in

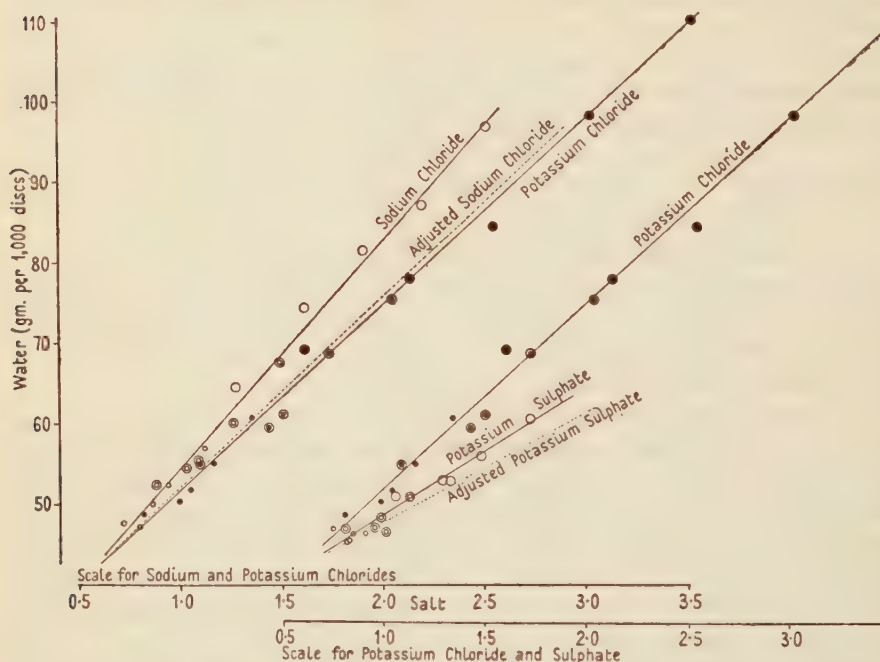


FIG. 4. Actual values for water and salt of discs floated on potassium chloride, sodium chloride, and potassium sulphate solutions. Regressions calculated from actual values are shown by continuous lines, while regressions calculated from values adjusted for difference in equivalent conductivity are shown by broken lines. Concentrations of external solutions are indicated by different circles.

Table IV. Now over the range of concentrations found in the sap the molecular lowerings of sodium and potassium chlorides are almost identical, sodium chloride being slightly higher in the more concentrated solutions. We have, however, compared the *water equivalents* of these two salts on the solute basis without any adjustment. Now potassium sulphate is much less effective in lowering the freezing-point, on the grammes equivalent basis, than the chlorides, and so total solute for the sulphate is an underestimate when compared with the chloride. Over the concentration range found, the lowering due to equivalents of chloride and sulphate was in the ratio of 1:0.645,¹ so that the increases of total solute due to sulphate have been raised for comparison purposes by this ratio, and a new *water equivalent* calculated on this basis. The results are shown in Table IV.

It will be seen that on either the salt or the solute basis, potassium and

¹ From 'Physikalisch-Chemische Tabellen', Landolt-Bornstein, Berlin, 1923.

sodium (as chlorides) have closely similar *water equivalents*, and it should be noted that in the second case, no adjustments are involved. On either basis the sulphate has a lower *water equivalent* than the chloride, and the difference is intensified on the adjusted basis. The difference between the adjusted chloride and sulphate *water equivalents* is of the same order, whether salt or solute is used as the basis of calculation.

TABLE IV
Correlation Coefficients between Water and Salt and Water and total Solute, and also Water Equivalents, on actual and on adjusted Bases, calculated from 'Total Salt' and 'Total Solute' Data for Potassium Chloride, Sodium Chloride, and Potassium Sulphate

	Correlation coefficients		Water equivalents calculated from			
	Water and salt.	Water and solute.	'Total Salt'		'Total Solute'	
			Actual.	Adjusted.	Actual.	Adjusted.
Potassium chloride	+0.996	+0.995	22.9	22.9	0.449	0.449
Sodium chloride	+0.996	+0.994	28.6	23.9	0.440	0.440
Potassium sulphate	+0.971	+0.937	15.5	13.7	0.367	0.237

A further experiment was carried out comparing the effects of sodium and calcium chlorides. The actual *water equivalents* based on unadjusted salt values were 29.6 for calcium and 32.6 for sodium. The difference between the two regressions reaches the level of partial ($P = 0.10$) but not full ($P = 0.05$) significance. Now although we ourselves have made no determination of the equivalent conductivities of sodium and calcium chlorides, tables show that calcium chloride has a slightly higher equivalent conductivity than sodium chloride. If our results had been corrected for this difference the *water equivalents* of the two salts would have been closer than on the actual basis. If there is a difference between the *water equivalents* of sodium and calcium chlorides, it is below the level of significance.

To sum up, it would appear that while there may be well-marked specific effects for anions, this is not the case for cations. The results suggest that salts affect hydration by acting in a colloidal rather than in a purely aqueous medium, and consequently that the *amount of salt rather than the concentration* is effective in water absorption.

C. Conclusion.

No evidence of a specific effect on hydration was detected when comparisons were made between sodium and potassium and between sodium and calcium. Marked differences were, however, found between sulphate and chloride.

IX. INERTNESS (Experiment 2I)

A. Introduction.

Leaf discs from many different plants have been floated on salt solutions. Some swelled as readily as cotton, others showed more limited swelling,

while still others did not swell at all. The monocotyledons as a group do not swell, but as they tend to show rapid injury they do not make suitable experimental material. The most satisfactory non-swelling material so far investigated is citrus which, owing to the non-wettable nature of the cuticle, floats very easily. Cabbage, cauliflower, and lettuce discs all swell readily but form poor experimental material because it is difficult to obtain flat discs. An experiment is described below in which the behaviours of four common West Indian plants are compared.

B. Procedure.

Discs were cut from cotton, rough lemon (*Citrus limonum*), egg plant (*Solanum melongena*), and cocoa (*Theobroma cacao*) leaves. In all cases young leaves approaching full size were used. They were floated in the normal manner (i.e. underside up) on water and on calcium chloride solution. In addition to the Initial collection, there were collections after 2, 5, 8, and 12 days. The discs from cocoa and egg plant showed considerable damage at the time of the last collection and so no results are given for these plants at that collection. Water and salt were determined in the usual manner.

C. Results.

The percentage changes in water and salt for the four plants are shown in Fig. 5. The results for the discs on calcium chloride are shown in the upper part of the figure. Cotton took up the most salt and water, and was followed by egg plant. Citrus discs took up salt readily—in fact, conductivity rose faster than in cotton—but there was no net uptake of water. There was in fact a slight but significant loss in water content initially, but this, however, was regained later. Cocoa discs took up very little salt and showed a small, steady loss in water content. It may be significant that the plants that swelled readily had higher initial water contents than those that did not swell. These contents for egg plant, cotton, citrus, and cocoa were 488, 379, 304, and 190 gm. water per 100 gm. dry weight.

The results for the discs floated on water are shown in the lower part of the figure. The water contents of cotton, citrus, and cocoa dropped slowly in time, the maximum change being in citrus, which lost 12 per cent. of water in 12 days. Egg plant showed a slight initial loss and then remained steady. Salt changes were small and showed no drift in time for cotton and citrus, while egg plant and cocoa showed a steady loss, reaching 27 per cent. after 8 days in cocoa.

In the case of citrus it seems clear that the absence of swelling was not due to inability of the discs to absorb salt, for they did absorb considerable quantities, the salt content being almost trebled. Furthermore, although salt did not cause any increase in water content over the initial value, it did prevent the loss that occurred in the water content of the Water treatment, and it did cause a slight but significant uptake of water in the discs on salt solution which compensated for the loss between the Initial and first collec-

tions. Salt seems to have played some part in the water relations of the discs but to have been incapable of causing any distension of the cell.

Plasmolytic tests were carried out on the citrus discs at the time of the third collection. Freehand transverse sections were cut and immersed in

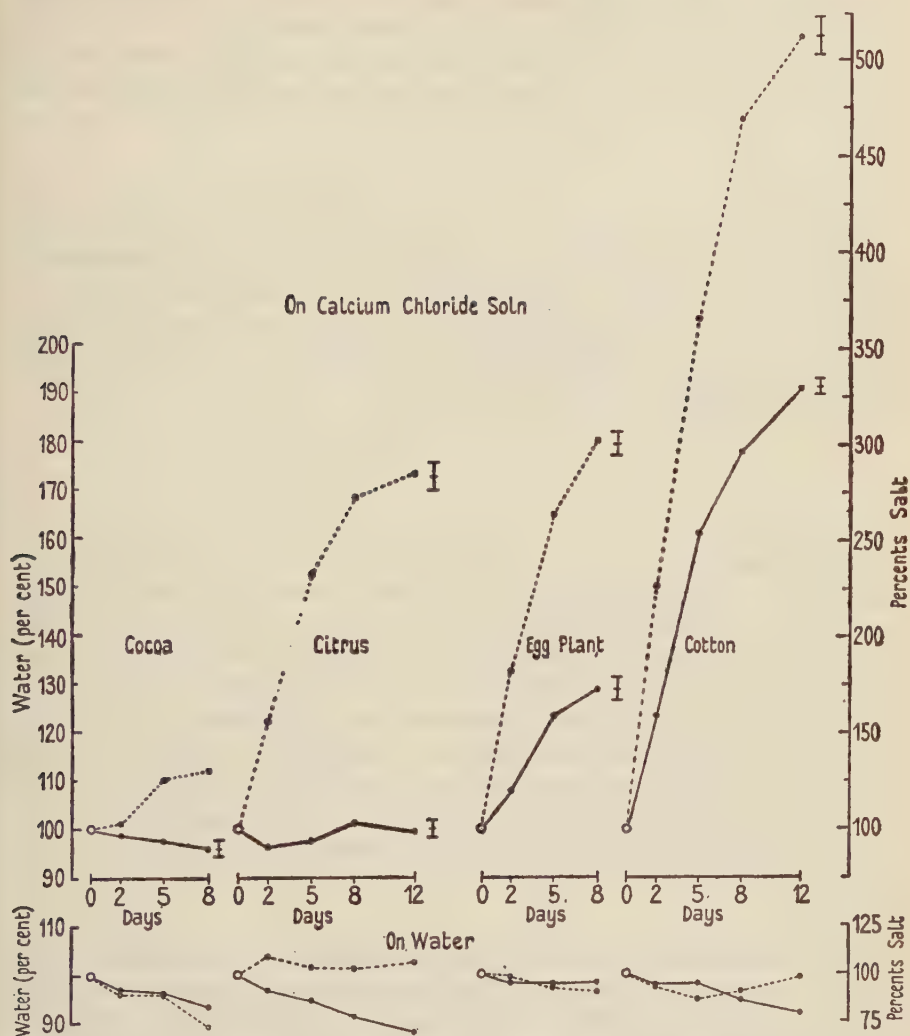


FIG. 5. Relative changes in salt (broken lines) and in water (continuous lines) of discs from cocoa, citrus, egg plant, and cotton floated on calcium chloride solution (above) and on water (below).

calcium chloride solutions of varying concentrations containing neutral red. The cells of the spongy mesophyll of citrus leaves are packed more closely than those of cotton and are more regular in shape. These regular parenchyma cells in the spongy mesophyll were used for plasmolysis. The 'osmotic

value' at incipient plasmolysis is taken as that concentration which causes definite plasmolysis in half the cells examined (cf. Bennet-Clark, Greenwood and Barker, 1936). The value for the discs on water was 0.4 M, while that for the salt discs was 0.7 M. Using osmotic terminology, it is clear that the 'osmotic value' at incipient plasmolysis of the citrus discs on salt had greatly increased. There was, however, no demonstrable water uptake. The results suggest that in citrus the cell wall or the protoplasmic framework or both are inextensible under the influence of salt, and we have termed this phenomenon 'Inertness'.

D. Conclusion.

Discs punched from leaves of some plants may absorb considerable amounts of salt and yet fail to absorb water. Such a plant is *Citrus limonum*. When floated on calcium chloride, discs show a considerable increase in 'osmotic value' at incipient plasmolysis over discs floated on water. It is suggested that, unlike cotton, the extensibility of the cell wall or that of the protoplasmic framework is unaffected by salt.

X. CONDITIONING (*Experiment 19, Part 1*)

A. Introduction.

We have attempted to make discs from citrus leaves swell by substituting other salts for calcium chloride, but without success. The use of growth hormones has also proved ineffective. On the other hand, attempts to make cotton inert like citrus have met with partial success. An experiment will now be described in which cotton discs were so *conditioned* that their responsiveness to salt (i.e. *water equivalent*) was diminished. The general procedure was to compare the salt/water relation of discs floated on salt solutions immediately on punching with that of discs transferred to salt solutions after floating on water for some time. The *conditioning* process thus consisted in checking growth by floating on water for some days.

B. Procedure.

This experiment is considered in two parts. In Part 1, which is considered in the present section, discs were floated on water and on calcium chloride solutions. Inspection of Fig. 6 will make the procedure clear. The discs floated on salt solutions immediately constitute the Normal series, while the discs transferred to salt solutions after 8 days on water constitute the Conditioned series. The discs which remained on water will be termed the Water series. The times of the collections are shown on the graph. Discs that have been cut and floated on water for some days before floating on salt solutions do not accumulate salt as rapidly as discs floated on salt solutions immediately. To compensate for this, higher concentrations of calcium chloride were used for the Conditioned series. The results as usual are calculated on the basis

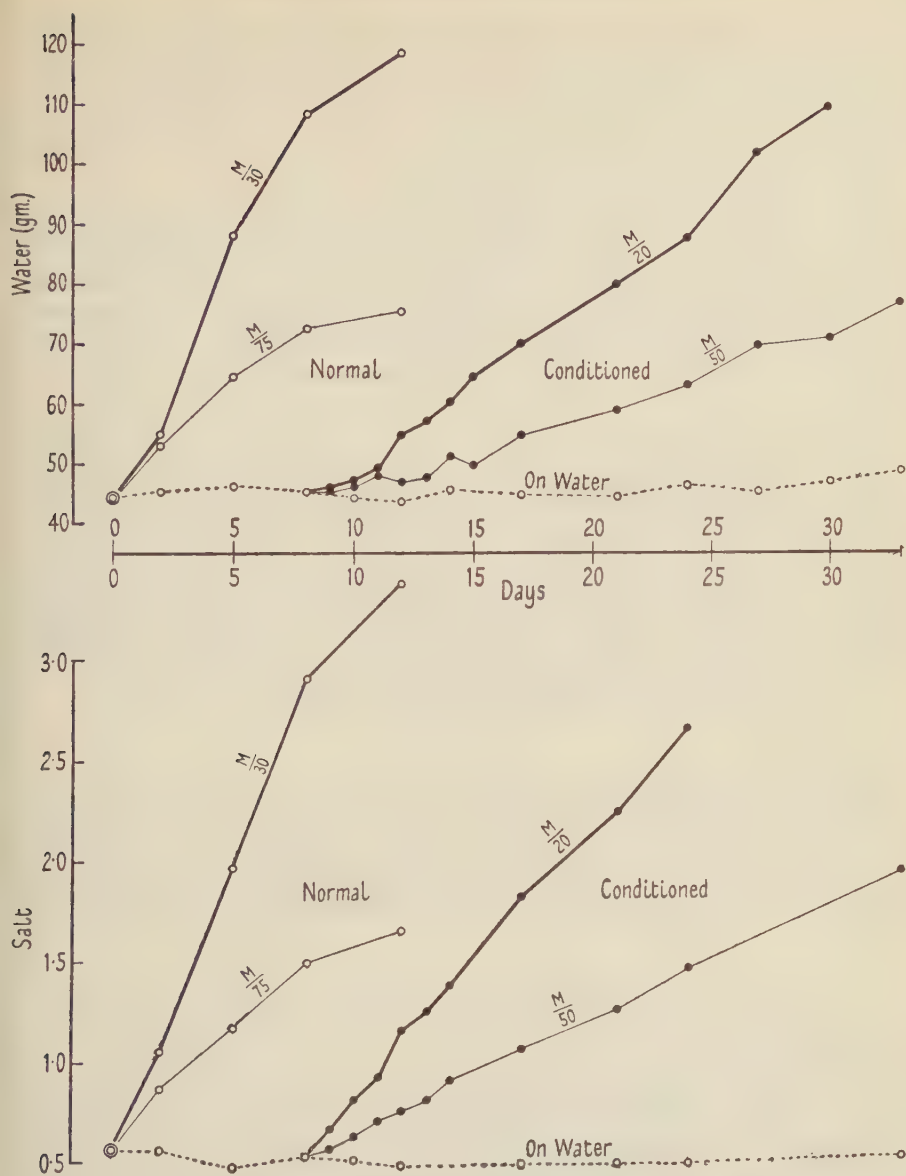


FIG. 6. Actual values for water (above) and salt (below) per 1,000 discs of normal and conditioned series floated on water and on calcium chloride solutions.

of 1,000 discs. The discs throughout were exposed to the normal diurnal alternation of light and darkness.

C. Results.

The changes in water and salt during the course of the experiment are shown in Fig. 6. Salt entered the discs of both the Normal and Conditioned

series without any appreciable initial lag. The rate fell off after the 8th day in the Normal series and remained steady in the Conditioned series until the end of the experiment. The rates of salt uptake were generally greater in the Normal than in the Conditioned series. The rate on the high concentration (M/20) of the Conditioned series was, however, greater than that on the low concentration (M/75) of the Normal series.

If we compare the rates of water uptake of the Conditioned and the Normal series, we see a marked difference during the first few days. This difference consists of a marked initial lag in water uptake in the Conditioned and not in the Normal series. *Conditioning* the discs has not, however, resulted in *inertness* such as we found in citrus, but only in a diminished responsiveness (i.e. *water equivalent*) especially during the first few days on salt.

When water is plotted against salt as in Fig. 7, it will be seen that there is not only an initial lag in water uptake in the Conditioned series, but that in addition to this initial difference, the Conditioned series showed a smaller *water equivalent* than the Normal. In both series, water and salt are very highly correlated ($r = +0.993$ in the Normal and $+0.997$ in the Conditioned series). In calculating the correlation coefficient for the Conditioned series the results for the first 3 days have been excluded. The regression coefficients for water on salt (i.e. the *water equivalents*) were 26.6 for the Normal and 21.5 for the Conditioned (excluding the results for the first 3 days).

D. Conclusion.

Discs were *conditioned* by floating on water for 8 days. They were then transferred to salt solutions and were compared with discs put immediately on to salt solutions. It was found that this *conditioning* process led to a sensible reduction in the *water equivalent*, especially during the first few days.

XI. CARRY-OVER (*Experiment 19, Part 2*)

A. Introduction.

In an earlier section we showed that most of the salt absorbed by discs was taken up during the day while water uptake went on steadily by day and night (cf. Fig. 2). The continued uptake of water after salt has ceased to enter we termed *carry-over*. The present experiment (Part 2 of expt. 19 described in the previous section) is concerned with *carry-over*. The uptake of salt was stopped by transferring the discs from the salt solutions to water and subsequent changes in the water content of the discs were then determined. It will be evident that this transfer from a salt solution to water might, quite independently of what we have termed *carry-over*, lead to an increase in the water content of the disc.

A priori considerations, however, suggest that this uptake of water caused by the release of back-pull would be small. Thus a calcium chloride solution

in the region of half-molar causes incipient plasmolysis and this is accompanied by a reduction in volume of about 5 per cent. When transferred to water the cells revert to their original volume. We may take it then that a

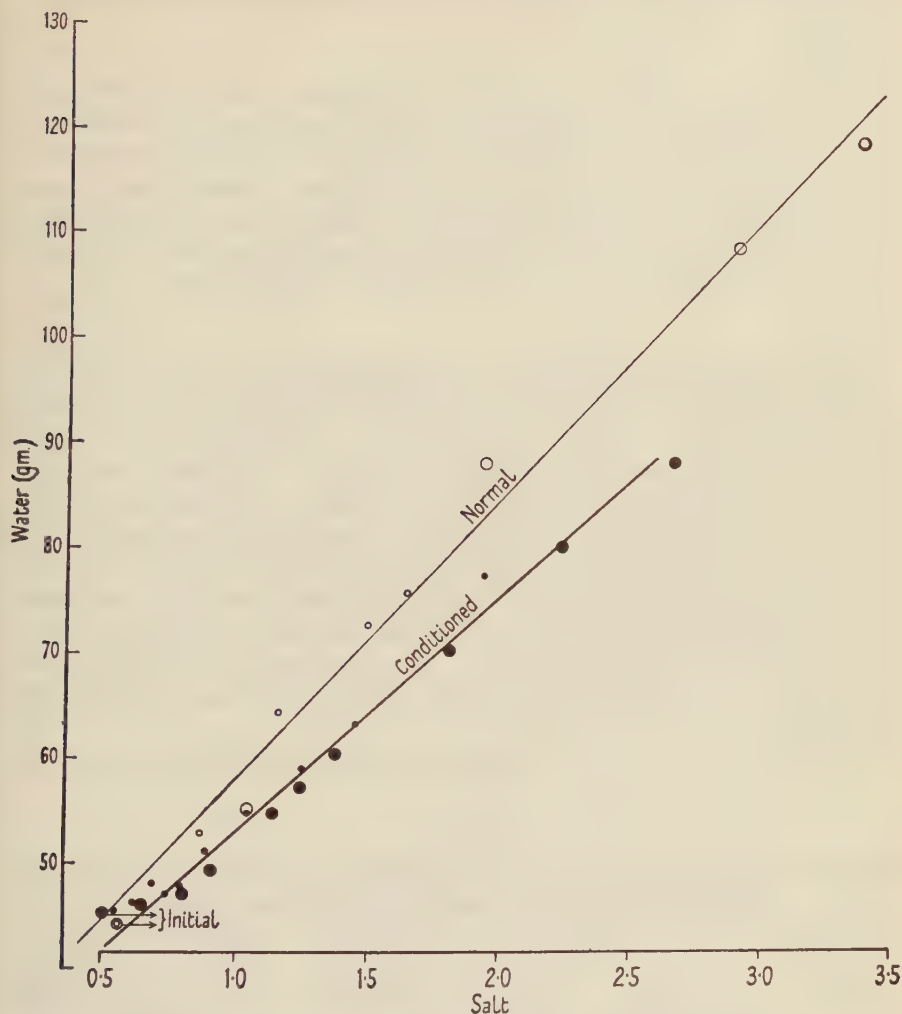


FIG. 7. Relation between salt and water in Normal (circles) and Conditioned discs (black discs). The two concentrations of calcium chloride used are represented by different sizes of circles and discs. The regression lines of water on salt are shown.

change in external concentration of 0.5 M causes a volume change of about 5 per cent. Transfer of discs from a M/30 solution of calcium chloride to water¹ would therefore cause a volume change of only 10/30 or 1/3 per cent.

¹ In this connexion it must be recollected that when discs are taken off salt solutions for moisture determinations they are well washed in several changes of water and remain wet for about 20 minutes. Thus discs on which moisture is determined should be in equilibrium with water, even though they were floating on salt solutions.

That the effect of the solution on which the discs were floated on the water content of the discs is negligible is also indicated by the results shown in Fig. 3. In this figure a linear relation is indicated between salt and water irrespective of the variation in the concentration of the external solution.

B. Procedure.

The procedure for Part 1 of this experiment has already been described. There were a Normal and a Conditioned series. In each series there were two concentrations of calcium chloride. The procedure in Part 2 consisted in transferring at each collection samples of discs from the salt solutions on to water and then determining the changes in moisture content at intervals of 3 days for a period of 9 days. The discs at the time of transfer were as usual well washed in water before drying for moisture determination.

C. Results.

In Fig. 8 are shown the changes in water content of the discs of the Normal series (M/30) and the Conditioned series (M/20). The changes in moisture content after transfer to water are also shown. The results for the Normal (M/75) and Conditioned series (M/50) are not shown in the figure. Inspection of the figure will make it clear that after transfer there were significant uptakes of water in the Normal (M/30) discs. At the transfers made on the 2nd and 5th days equilibrium appears to have been established after 3 days on water. At the two subsequent transfers equilibrium appears to have been established later. In the Conditioned series equilibrium on the whole appears not to have been established till about the 6th day. It will be noticed that in this series the extent of the *carry-over* was greater at the later than at the earlier collections.

In Table V are shown the maximum amounts of water taken up by the

TABLE V

Maximum Water Increases (actual and relative) on transferring from Salt Solutions to Water in Normal and Conditioned Series

Days on salt solution.		Actual increase (gm.).		Relative increase.	Actual increase (gm.).		Relative increase.
2	Normal series	3.63	M/75	6.9	8.04	M/30	14.6
5		3.46		5.4	7.51		8.5
8		1.92		2.6	6.05		5.6
12		2.74		3.6	8.19		6.9
1	Conditioned series	1.67	M/50	3.7	2.23	M/20	4.8
2		2.03		4.4	4.89		10.4
3		1.95		4.1	8.16		16.5
4		4.54		9.7	4.84		8.8
5		4.10		8.6	7.68		13.4
6		3.66		7.2	8.41		13.9
9		3.49		6.4	13.07		18.6
13		6.85		11.6	17.78		22.1
16		4.24		6.7	18.31		20.8

discs after their transfer to water, i.e. *carry-over*. The percentage values are also shown in the table. It will be seen that in both series the *carry-over* was greater on the high concentrations than on the low. It will also be seen that

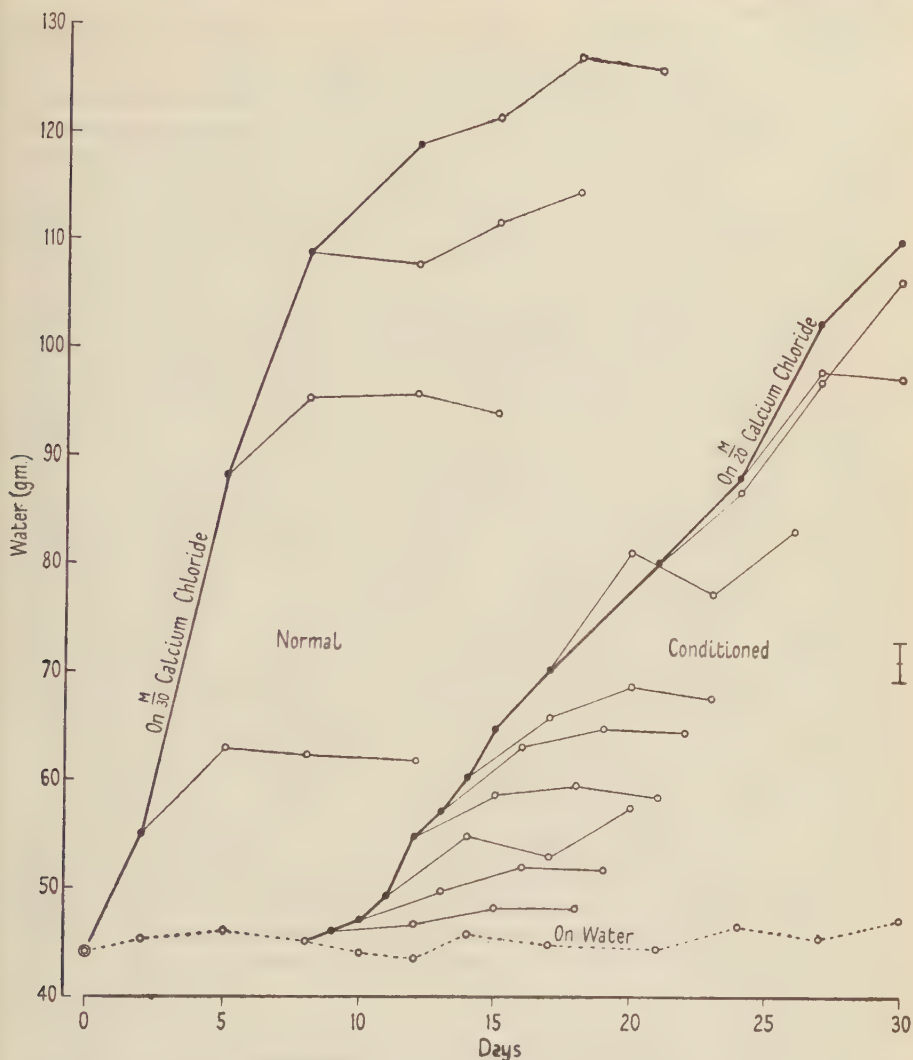


FIG. 8. Changes in water content of Normal and Conditioned discs transferred from salt solutions to water. The water values for discs at the time of transfer to water are shown by black discs and subsequent water values by circles.

in the Conditioned (M/20) series the *carry-over* on two occasions actually exceeded 20 per cent. In the Normal series the *carry-over* does not appear to have increased in time, while in the Conditioned, an increase in time is quite marked.

We will now revert to Part I of the present experiment. In Fig. 7 is shown

the relation between water and salt for the two series. It was pointed out that the two series have quite different *water equivalents*. Thus, the regression coefficients for water on salt were 26.6 for the Normal and 21.5 for the Conditioned series.

If instead of the actual water content at the time of transfer we substitute the maximum moisture content attained after transfer to water, we obtain the results shown in Fig. 9. In this figure the salt content at the time of transfer is plotted against the maximum water content after the discs were transferred to water. The actual salt contents corresponding to the maximum water contents were not determined. It will be seen that the difference between the two series shown in Fig. 7 has now virtually disappeared. The *water equivalent* for the Normal series has only been increased from 26.6 to 28.8, while that for the Conditioned has been increased from 21.5 to 32.2.

These results suggest that the *potential water equivalent* is approximately the same in the two series and that the effect of *conditioning* is to retard the attainment of the full hydration value of salt. Thus in the Normal series the actual water values are nearer to the potential water values than in the Conditioned series (cf. Fig. 8). In short, salt took longer to exert its full effect on hydration in the Conditioned than in the Normal series.

It seems unlikely that this is due to a reduction in permeability to water on *conditioning*, for when Conditioned discs are wilted the full water content is regained in a matter of hours just as with Normal discs. As we have seen, when discs are transferred from salt to water it takes days to reach equilibrium. If a change in permeability to water is not the explanation, it may be that salt takes longer to influence cell extensibility in Conditioned than in Normal discs. It will also be evident that some of the variation noted in the *water equivalents* (see section VII) may have been due to variations in the rate of cell extensibility in different experiments (i.e. variations in *conditioning*).

Before closing this section attention should be drawn to the difference in the forms of the Conditioned curves before and after correction for *carry-over*. In Fig. 9 (inset) salt has been plotted against the actual water values and against the potential water values for the first few days the discs were on salt. It will be seen that the initial lag shown when the actual values are used is ironed out when these values for water are replaced by the potential values.

D. Conclusion.

Discs transferred from salt solutions to water continue to take up water for several days. This continued uptake of water has been termed *carry-over*. It may exceed 20 per cent. of the water content of the disc at the time of transfer from salt to water. It is not apparently due to the release of back-pull from the salt solution.

The maximum water content attained on water has been termed the potential water content, while the water content at the time of transfer from the salt solution has been termed the actual water content. *Carry-over*, therefore, is equal to the difference between the actual and potential water contents.

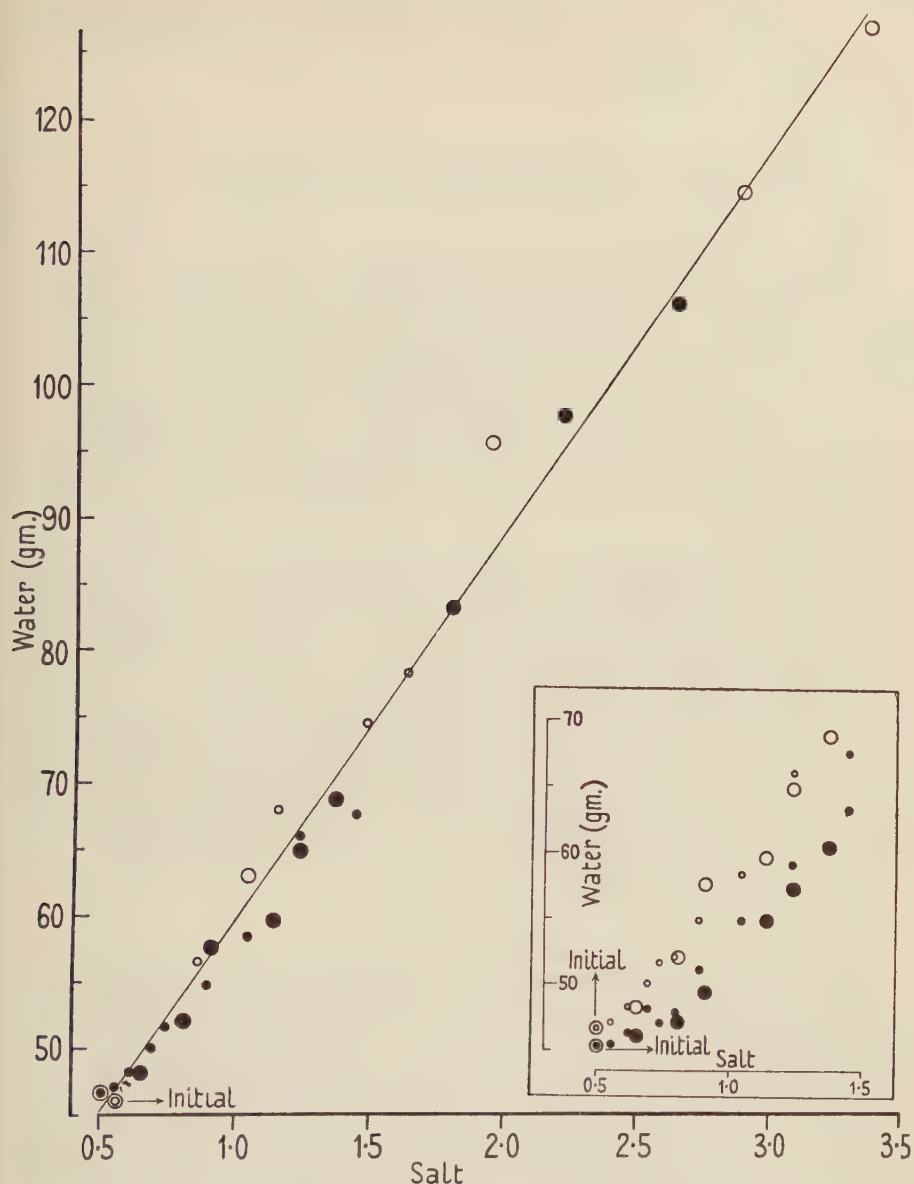


FIG. 9. Relation between salt and potential water in Normal (hollow circles) and Conditioned discs (solid circles). The two calcium chloride concentrations used in each series are shown by different sizes of circles. INSET: The relation between salt and water in Conditioned discs. Actual water (discs) and potential water (circles) are shown.

The *water equivalents* of Normal and Conditioned discs are markedly different when the actual moisture contents are considered. On the other hand, the *water equivalents* of Normal and Conditioned discs are almost identical

when the potential moisture contents are considered. The effect of *conditioning*, therefore, is to retard the rate at which the full saturation capacity of salt is satisfied. It is suggested that this is due to a diminution in the rate of extensibility.

XII. SWELLING ON NUTRIENT SOLUTIONS (*Experiment 22*)

A. *Introduction.*

Previous work (Mason and Phillis, 1943) led us to suggest that the water content of the cell was determined by the quantity of protoplasm and that the hydration capacity of protoplasm was markedly influenced by its salt content. In all disc experiments so far described in this paper we have tacitly assumed that the amount of protoplasm remained constant throughout each experiment, so that the changes in moisture content could be attributed to changes in hydration capacity. In this section we present an experiment in which we attempted to change the moisture content of the disc by changing the amount of protoplasm.

We (Mason and Phillis, 1942*a*) have already described an experiment in which discs were floated on a nutrient solution similar to that normally employed for growing plants in sand and water culture. We reported that after a sharp initial rise in sap conductivity there was no further change but that the water content of the disc continued to rise. Though there occurred a marked increase in water without a rise in conductivity, there did occur an increase in salt. In the present experiment we have attempted to minimize this rise in salt by excluding all non-essential elements and by greatly reducing the bases calcium and magnesium. It was thought that by so doing the influence of protoplasm, or perhaps it would be more correct to say non-salt factors, might be more readily revealed.

B. *Procedure.*

The discs were floated on water and on a nutrient solution of the following composition: 300 p.p.m. of nitrogen (ammonium nitrate), 50 p.p.m. of phosphorus, 150 p.p.m. of potassium, 25 p.p.m. each of calcium and magnesium, 33 p.p.m. of sulphur; traces of iron, manganese, aluminium, zinc, copper, and boron were added. Sodium and chlorine, which are usually present in our nutrient solutions, were omitted. The usual concentrations of potassium, calcium, and magnesium employed are 200, 100, 100 p.p.m. respectively. The conductivity of the new, low-salt solution was reduced to approximately 60 per cent. of the normal value.

The experiment continued for 15 days and there were 5 collections in addition to the Initial. At each collection conductivity was determined on the expressed sap, protein after alcohol extraction of the fresh material, while nitrogen, phosphorus, calcium, and ash (sulphated) were determined on the dried material.

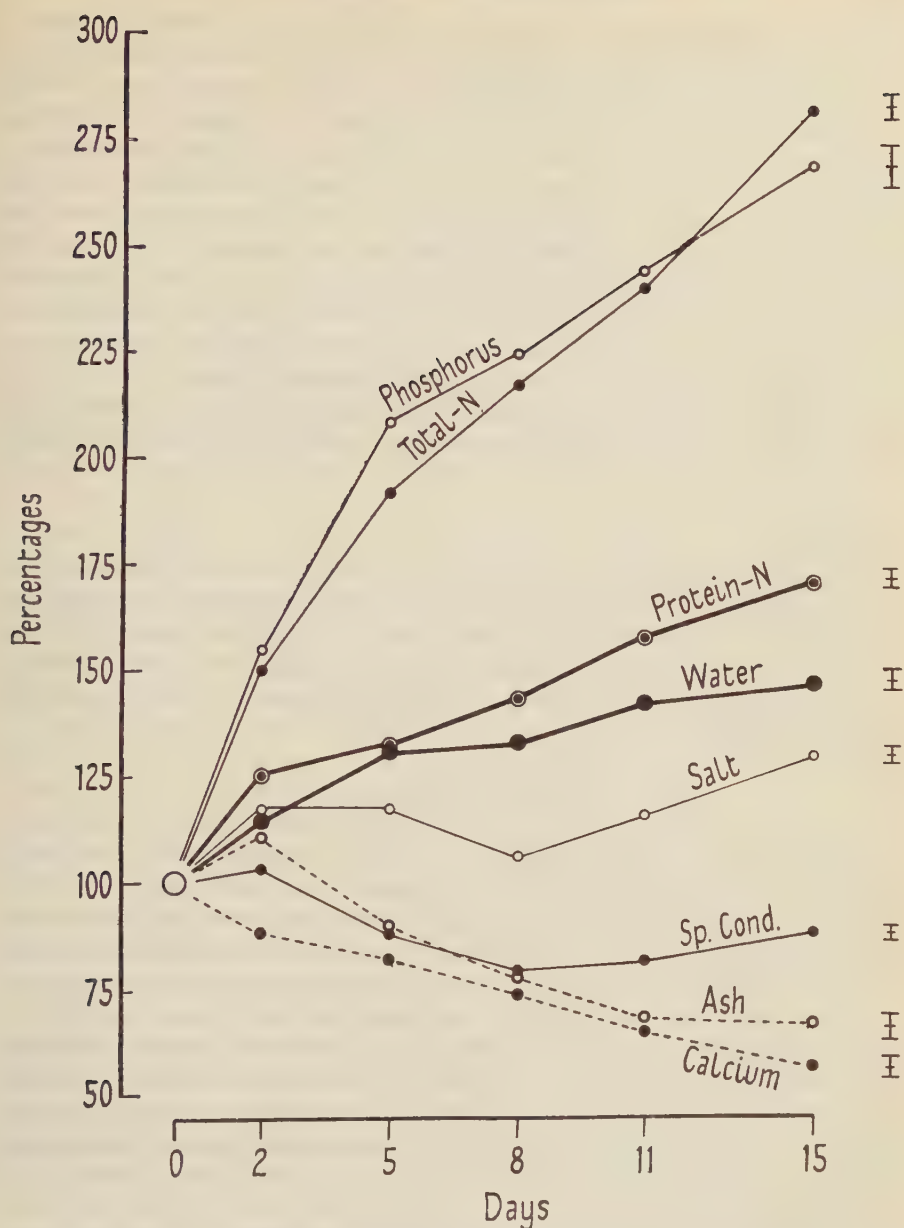


FIG. 10. Relative changes in phosphorus, total-N, protein-N, water, salt, specific conductivity, calcium, and ash of discs floated on a full nutrient solution.

C. Results.

The results for the discs floated on water are not presented as they showed no unusual features. The results for the discs floated on the nutrient solution are shown in Fig. 10. All results are shown as percentages of the initial value.

It will be seen that there was quite a marked increase in water, a decrease in conductivity, and very little change in salt except between the Initial and first collections. It seems clear that the change in water is quite independent of the change in salt. There were marked losses in calcium and in ash from collection 1 onwards, and there were great increases in nitrogen and phosphorus. The changes in water were most closely paralleled by those in protein. The results as they stand suggest that protein or a combination of protein and phosphorus in new protoplasm was the cause of the increase in water content. It is, however, far from clear why there were substantial losses of calcium and ash. If exchange with ammonium was responsible, then it is perhaps surprising that the discs showed no injury. It must also be recollected that while conductivity may be a safe guide to changes in concentration when discs are floated on pure salt solutions, the case is quite otherwise when a complex mixture such as was used in the present experiment is employed.

D. Conclusion.

Discs floated on a full nutrient solution showed a marked increase in water content with no parallel increase in salt and with losses in calcium and ash. The moisture changes were very similar to the changes in protein. While at first sight this suggests the presence of non-salt factors affecting water content, difficulties in interpreting conductivity measurements in complex mixtures confuse the issue.

XIII. DISCUSSION

Ponder (1944) remarks that 'A survey of the results of the experiments which have been done on the volume changes of mammalian red cells in solutions of various tonicities shows that the red cell sometimes behaves as a perfect osmometer, while at other times it does not'. He found that red cells of human oxalated blood in hypotonic plasma undergo anomalously small swelling. He attributed this to the existence of elastic forces sufficient to resist osmotic swelling. These elastic forces are assumed to reside in the gel framework of the protoplasm of crenated cells, while normal cells are assumed not to be gelled. He regards crenation of the red cells as corresponding to gelation. He found that the bulk modulus for crenated cells, calculated from measurements of swelling in hypotonic plasma, is of the same order as that for gelatin gels.

It is generally assumed by botanists that such structural (protoplasmic) forces play no part in cell-water relations. Osmotic forces exerted inside the large central vacuole are assumed to be opposed only by elastic forces resident in the cellulose wall. This is probably due to the fact that the protoplasm is usually assumed to consist only of a thin perietal layer and to function merely as a semipermeable membrane. If, however, we are correct in our belief that in the foliar cell of cotton the protoplasm occupies about

70 per cent. of the volume of the cell and the central vacuole only about 90 per cent., then the question arises whether these structural forces in the protoplasmic gel may not play a highly important role in resisting osmotic distension of the cell.

Before we attempt to explain the mechanism of salt hydration a word should be said concerning the interrelation of the central vacuole and the minute vacuoles assumed to be present in the meshwork of the protoplasmic framework. As we have pointed out, both appear to swell when salt enters the cell. Not only do they appear to swell, but they appear to swell to about the same extent. Now the osmotic concentration in the vacuole is only about one-tenth of that in the cytoplasmic vacuoles. The osmotic pressure in the central vacuole is only about 2 atmospheres, while that in the cytoplasm may be as much as ten times as great. How can these differences be explained? When the cell is in equilibrium with water, then the osmotic pressure in the central vacuole will be equal to the wall pressure in the tonoplast,¹ while the osmotic pressure in each cytoplasmic vacuole will be equal to the wall pressure in the protoplasmic framework surrounding it. Equilibrium, then, will be maintained by the differences in the wall pressures of the tonoplast and the protoplasmic framework. Physically, then, the central vacuole and the cytoplasmic vacuoles will differ in that hydrostatic pressure in the central vacuole is much less than in the cytoplasmic vacuoles, and its volume much greater.

One of the most remarkable features of the swelling that takes place when discs are floated on salt solutions is its magnitude. In one experiment we found that discs punched from apparently mature leaves increased their water content about three times within a period of a month. A priori, water might enter the cell either as a result of an increase in the forces inside the cell that attract water or as a result of a diminution in the forces that oppose swelling. Thus an increase in osmotic forces followed by an elastic or plastic extension of the restraining structures or else a weakening of the restraining structures without any increase in osmotic pressure would lead to an increase in the water content of the cell.

The first possibility must be rejected as the cause of salt swelling for the following reasons. We found that an increase in the osmotic pressure of the sap of the order of 2 atmospheres, due to an increase in sugar concentration, was not accompanied by any appreciable uptake of water. We have also shown that water uptake may occur without a corresponding increase in the concentration of electrolytes. Moreover, we found that chlorides and sulphates differ in their *water equivalents*. It must also be recollected that very large forces bring about only very small changes in the water content of the cell (cf. section XI) or, in other words, that the cell structures restraining swelling require very large forces before they allow an appreciable amount of water to enter the cell.

Now though water uptake may take place without an increase in the concentration of electrolytes, it is always accompanied by an increase in the

¹ The wall pressure on the cellulose wall will of course be less than that on the tonoplast.

amount of salt. The question arises, therefore, whether salt *per se* may not in some way weaken the structures restraining swelling so that they become extensible and allow osmotic forces to pull water into the cell. A clue, perhaps, is provided by the absorption of water by gelatin. In the iso-electric region an increase in salt allows water to be taken up by the gel. According to Jordan Lloyd and Pleass (1927), 'The water content of the jelly is fixed by a balance between osmotic forces in the sol and the elastic forces in the gel framework, the latter also containing water present as a solid solution. Reactions which increase the osmotic forces in the interstitial fluid or those which weaken the elastic forces of the framework lead to swelling.' They suggest that salts may weaken the framework and the elastic recoil. It may be that salts similarly weaken the protoplasmic framework and thus increase the pressure on the cellulose wall.

It is not clear how this increase in pressure on the cellulose wall would increase its extensibility. *Elastic* extension must be rejected, for the reduction in volume from full turgor to incipient plasmolysis is similar in tissues before and after salt-swelling. Plastic extension is of course possible, as is the possibility of vital activity in the cell wall (cf. Anderson, 1935). In this connexion we have found that in discs that were *salt-swollen 50 per cent.*, the amount of cellulose¹ had increased 25 per cent., while in control discs floated on water the cellulose content increased by only 14 per cent.

If salt causes the cell to absorb water by weakening the protoplasmic framework, the question arises as to what changes take place during *conditioning*. *Conditioning*, as we have stressed, does not alter the potential *water equivalent*. It only retards the rate at which water equilibrium is attained. It may be suggested that *conditioning* consists in a strengthening of the protoplasmic framework; possibly it becomes denser so that though the bonds are loosened rapidly by salt the mechanical movement due to the osmotic intake of water takes longer. In the case of 'inert' plants, such as citrus, which accumulate salt but fail to absorb water, it may be that the same is true and that the framework is so strong that the osmotic forces cannot distend it. It is also possible that the protoplasm is not gelled so that there is no framework on which the salt can act.

The linear correlation between salt and water which is shown in all our experiments except at very high salt levels might suggest a stoichiometric relation between salt and water. The differences in the *water equivalents* of different experiments might disappear if the potential and not the actual water values were known. A stoichiometric relation would suggest the existence of water of hydration in the protoplasm. In a recent paper (Mason and Phillis, 1939) we stressed this possibility. The hydration capacity of the protoplasmic proteins would on this view be determined by the amount of salt, and the extensibility of the cellulose wall would be determined by the hydration capacity of intermicellar protoplasm. Water of hydration would replace free water in the interstitial spaces. *Conditioning* would again be due

¹ Determined by the method of Chen and Cameron (1942).

to a strengthening of the protoplasmic framework and *carry-over* to the time taken by hydration water to distend the framework. The water in the central vacuole is of course free water, and osmotic forces would lead to the distension of the framework.

XIV. SUMMARY

1. The relative distribution of water between vacuole and cytoplasm remained unchanged when cotton leaves attached to the plant were caused to swell under the influence of salt solutions applied to the roots.

2. The uptake of salt by leaf discs floated on salt solutions is greatly accelerated by light. Salt uptake in the dark is negligible.

3. Water uptake in response to salt uptake appears to be independent of light, and continues for some days after salt uptake has ceased. This persistence in water uptake has been called *carry-over*.

4. The rate at which water is taken up in response to salt accumulation can to some extent be controlled by preliminary treatment of the disc. Discs *conditioned* by floating on water for several days took up water more slowly when transferred to salt solutions than discs placed immediately on salt solutions. The amount of water finally taken up did not, however, differ significantly.

5. Osmotically active non-electrolytes such as sugar cause no significant swelling.

6. The relation between salt and water is linear over a considerable range.

7. These observations *are not* in harmony with the classical osmotic theory of cell-water relations, but *are* in harmony with the view that protoplasm possesses structure, and that this structure, like that of protein gels, can be weakened by salt.

8. Some leaves accumulate salt and do not take up water, e.g. citrus. In such cases it is suggested that the extensibility of the cell wall or of the protoplasmic structure or of both is unaffected by salt.

LITERATURE CITED

- ANDERSON, D. B., 1935: The Structure of the Walls of the Higher Plants. Bot. Rev., i. 52.
 ATKINS, W. R. G., 1916: Researches in Plant Physiology. London.
 BENNET-CLARK, T. A., GREENWOOD, A. D., and BARKER, J. A., 1936: Water Relations and Osmotic Pressures of Plant Cells. New Phyt., xxxv. 277.
 — and BEXON, D., 1939: Expression of Vacuolar Sap. Nature, cxliv. 243.
 BUZAGH, A., 1937: Colloid Systems. London.
 CHEN, W. W., and CAMERON, F. K., 1942: Cellulose Content of Cotton and Southern Woods. Journ. Ind. and Eng. Chem., Ind. Ed., xxxiv. 224.
 GREATHOUSE, G. A., 1938: Conductivity Measurements of Plant Sap. Plant Physiol., xiii. 553.
 HAYNES, A., 1919: Electrical Conductivity as a Measure of the Content of Electrolytes of Vegetable Sap. Biochem. Journ., xiii. 111.
 HOAGLAND, D. R., and BROYER, T. C., 1942: Accumulation of Salt and Permeability in Plant Cells. Journ. Gen. Physiol., xxv. 865.
 JORDAN LLOYD, D., 1938: Protein Structure and Water Absorption. Journ. Phys. Chem., xlii. 1.
 — and PLEASS, W. B., 1927: The Absorption of Water by Gelatin. Biochem. Journ., xxi. 1352.

- MASON, T. G., 1919: On some Factors Affecting the Concentration of Electrolytes in the Leaf Sap of *Syringa Vulgaris*. *Scient. Proc. Roy. Dub. Soc.*, xv. 651.
- and PHILLIS, E., 1936: The Concentration of Solutes in Sap and Tissue, and the Estimation of Bound Water. *Ann. Bot.*, l. 437.
- 1939: Experiments on the Extraction of Sap from the Vacuole of the Leaf of the Cotton Plant and their Bearing on the Osmotic Theory of Water Absorption by the Cell. *Ibid.*, N.S., iii. 531.
- 1942: The Effects of Potassium Supply and Size of Plant. *Ibid.*, vi. 443.
- 1942a: Preliminary Observations Using the Disc-Culture Method. *Ibid.*, 455.
- 1943: The Influence of Composition and Concentration of Nutrient Solution. *Ibid.*, vii. 157.
- MEYER, B. S., 1931: Effects of Mineral Salts upon the Transpiration and Water Requirement of the Cotton Plant. *Amer. Journ. Bot.*, xviii. 79.
- PEARSALL, W. H., and EWING, J., 1929: The Relation of Nitrogen Metabolism to Plant Succulence. *Ann. Bot.*, xliii. 27.
- PHILLIS, E., and MASON, T. G., 1940: Preliminary Observations on Potassium, Calcium, and Magnesium. *Ibid.*, N.S., iv. 773.
- 1941: On the Expression of Sap by Low Pressure. *Ibid.*, v. 15.
- 1942: On Diurnal Variations in the Mineral Content of the Leaf of the Cotton Plant. *Ibid.*, vi. 437.
- 1943: Preliminary Observations Using the Pruning Method. *Ibid.*, vii. 147.
- PONDER, E., 1944: The Osmotic Behaviour of Crenated Red Cells. *Journ. Gen. Physiol.*, xxvii. 273.
- RICHARDS, F. J., 1944: A Critical Examination of New Theories of the Metabolism of Major Nutritive Elements in Plants. *Ann. Bot.*, N.S., viii. 43.
- and SHIH, Sheng-Han, 1940: The Relationship between Water Content and Composition of Leaves. *Ibid.*, iv. 403.
- SEIFRIZ, W., 1936: *Protoplasm*. New York and London.
- 1942: *The Structure of Protoplasm*. Iowa.
- TRELOAR, A. E., 1936: *An Outline of Biometric Analysis*. Minnesota.

Studies on the Partition of the Mineral Elements in the Cotton Plant

V. An Adsorption Theory of Nitrogen Regulation¹

BY

T. G. MASON

AND

E. PHILLIS

(Cotton Research Station, Trinidad, B.W.I.)

With one Figure in the Text

I. INTRODUCTION

THIS is our fourth paper on the partition of nitrogen. In the first (1939) we showed that for the leaf the *partition index* (i.e. protein nitrogen as a per cent. of total nitrogen) in any one experiment was highly and negatively correlated with nitrogen per 100 gm. dry weight. The experiments considered included rather wide variations in the supplies of nitrogen, phosphorus, and potassium. The correlations were not, however, linear. We concluded that the extent to which the tissue was saturated with nitrogen was an important factor determining the partition of nitrogen.

In the second paper (1942) we showed that discs punched from leaves could readily form protein when supplied with inorganic nitrogen and concluded that the root can play no part in protein regulation in the leaf through the agency of a hormone as Chibnall (1939) suggested.

In the same paper we proceeded to consider regulation in a number of experiments, including a sand-culture experiment with varying nitrogen supply, a water-culture experiment in which nutrient concentration was varied, a sand-culture experiment in which the supplies of nitrogen, of phosphorus, and of potassium were each varied though not over wide ranges, and also a disc-culture experiment in which discs punched from leaves were floated on a high nitrogen solution. These experiments were chosen because they covered a considerable range of nitrogen and together supplied an overlapping chain of data from very low to very high nitrogen levels.

The results were pooled and considered in three different ways. First, the *partition index* was plotted against nitrogen per 100 gm. dry weight as in our first paper. The correlation was again negative and not linear. Secondly, we plotted the *partition index* against crystalloid nitrogen per 100 gm. dry weight. The correlation was linear and reached the very high level of -0.994 .

Thirdly, we plotted protein nitrogen per 100 gm. dry weight against crystalloid nitrogen also expressed per 100 gm. dry weight. The linearity of the

¹ Paper No. 35 from the Physiological Department of the Cotton Research Station, Trinidad.

relation between the *partition index* and crystalloid nitrogen enabled us to calculate, from the regression equation for these variables, the protein levels for various crystalloid nitrogen levels. The calculated curve relating protein nitrogen and crystalloid nitrogen was shown graphically along with the observed values. We concluded that 'considering the range the experiments cover, the agreement must be regarded as good'. As the crystalloid level increased, the protein level also increased until it reached a maximum value, after which further increases in the crystalloid level brought about a reduction in the protein level.

Buzagh (1937) lists several types of relation between concentration and adsorption, the *most common type* of apolar adsorption being one in which the amount of material adsorbed rises at first with increasing concentration, reaches a maximum, and then at still higher concentrations declines. As our data relating the protein and crystalloid levels exhibited much the same behaviour we 'tentatively suggested that adsorption plays a dominant role in determining the equilibrium between protein and crystalloid nitrogen'. Though the most common types of apolar adsorption reach a maximum and then decline, other types may apparently exhibit no decline. It may be added that the mechanism of apolar adsorption is not fully understood.

As no single experiment in our second paper covered the whole range of protein increase and decline, it was felt that the observed relation between protein and crystalloid nitrogen might conceivably have been due to the choice of the four experiments considered rather than to any common unifying relation. In our third paper (1943) we accordingly presented data covering the necessary range. The results again showed the type of relation characteristic of apolar adsorption. Moreover, the decline in protein level after the maximum had been reached was fully significant. It was concluded that the experimental values fitted very closely to the *curve* calculated from the regression equation relating protein and crystalloid nitrogen. Though the experimental values did on the whole fit very closely to the calculated curve, they exhibited, nevertheless, a somewhat different pattern in that the experimental values rose rather more rapidly than the calculated curve.

Richards (1944) has recently published some comments on the theory of apolar adsorption. We propose at present only considering his comments in so far as they relate to nitrogen. 'It appears doubtful', he says, 'whether the equilibrium concentration per unit volume is at all adequately represented by crystalloid nitrogen content.' We confess we are somewhat in the dark as to what exactly his meaning is. If he is suggesting that some particular crystalloid nitrogen fraction is involved and not the whole crystalloid fraction, he is possibly right; but the lability of the crystalloid fractions may well be considerable (see discussion).

Secondly, he inquires of what the adsorbing surface consists. He presumes it is not the protein itself nor any surface quantitatively dependent upon protein. We offer the suggestion that the surface is enzymic and is related to the amount of protoplasm rather than the amount of protein.

Thirdly, he refers to the difficulties involved in treating as one reactant the complex of soluble components of nitrogen. This seems to be the same point referred to in connexion with crystalloid nitrogen adequately representing the 'equilibrium concentration per unit volume'.

His fourth comment is also a little obscure. 'Of far greater importance', he says, 'is the employment of the partition indices.' As the resemblance between apolar adsorption and our results was judged only on the way the protein and crystalloid nitrogen values arranged themselves as in Fig. 1 of our second paper, he presumably had in mind our comments on *his own* data. Here we think he is quite correct and our conclusion, in so far as it rests on the use of the *partition index*, that his data are in harmony with apolar adsorption, must be withdrawn. We will refer to his experiments in the discussion.

Fifthly, he says: 'Neither do their own data supply altogether convincing evidence when the correlation diagram between the contents of the two nitrogen fractions is considered.' He referred to the results presented in Fig. 1 of our second nitrogen paper (1942). As the results presented in Fig. 1 of our third nitrogen paper (1943) must have removed his doubts concerning continuity, &c., we will not discuss further the data in Fig. 1 of our second paper (1942).

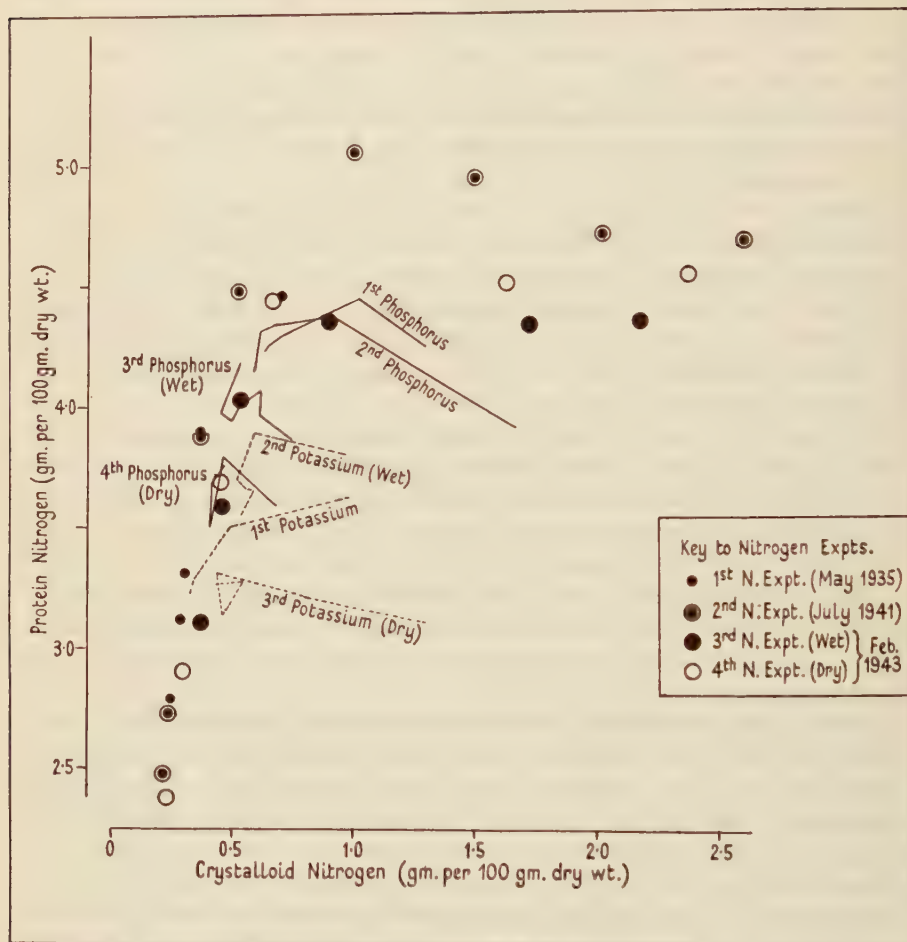
To sum up, we see no reason to modify our view that the results presented are in harmony with the view that nitrogen regulation *may* be controlled by some form of apolar adsorption. Now, our opinion does not rest only on the resemblance between the changes in the protein/crystalloid values in our experiments and the changes that occur in apolar adsorption, for it seems to us that for protein synthesis to take place the crystalloid fraction must be adsorbed either on the protein or on some other surface. How else, it may be asked, can the necessary contact be established between two reactants not in the same phase?

It will be evident that the fact that there is a maximum value for protein in an experiment merely indicates that there is some factor limiting protein synthesis. The factor need not necessarily be the amount of surface available, for it might well be the amount of some mineral element available. Thus Hoagland (1944), in commenting on the possible functions of potassium, remarks: 'since potassium is an essential element for plant growth it is ultimately necessary for every kind of synthesis or metabolism in the plant. The question at issue is, how direct a role does it play?' There is some evidence that potassium is involved in protein synthesis (cf. Wall, 1940). The question, as Hoagland says, is, 'how direct a role does it play?' Though it cannot presumably enter directly into the reaction, it might determine the amount of protoplasm and indirectly the area of the surface on which adsorption takes place. In the present paper we have attempted by means of experiments covering not only a wide range of nitrogen supply, but also of potassium and phosphorus supply, to learn something more about the factors controlling protein synthesis in the leaf.

II. THE EFFECTS OF VARIATIONS IN NITROGEN, PHOSPHORUS, AND POTASSIUM SUPPLIES ON THE PROTEIN LEVEL

A. Procedure.

We will describe the results of four nitrogen, three potassium, and four phosphorus experiments. In all these experiments the plants were grown in



Relation between Protein N and Crystalloid N per 100 gm. dry weight. The four nitrogen experiments are shown by circles and dots not connected, and the four phosphorus and three potassium experiments by connected lines.

small containers holding about 10 lb. of sand. Only leaves from vegetative plants were used.

For the first nitrogen experiment the procedure was described in our first paper (1939), while the second was described in our second paper (1942). The third and fourth followed the same procedure except that they were

carried out at the same time, one (i.e. the third experiment) in a greenhouse under humid conditions, and the other (i.e. the fourth experiment) in a wire-house under dry conditions. In addition to a difference in humidity there was also a considerable difference in light intensity. We will refer to the conditions as Wet and Dry respectively.

The first potassium experiment was described in our first paper (1939), while in the second and third the procedure was essentially the same, except that in the second experiment the plants were grown in a humid greenhouse and in the third in a dry wire-house. The second and third experiments ran concurrently.

The phosphorus experiments followed the same plan. The first, second, and third were carried out in greenhouses and the fourth in a wire-house. The first and second ran concurrently in greenhouses. The third and fourth experiments also ran concurrently.

B. Results.

The results for the eleven experiments are shown graphically in the figure. Protein nitrogen per 100 gm. dry weight has been plotted against crystalloid nitrogen also expressed per 100 gm. dry weight. The individual values of the nitrogen experiments have not been joined by lines. For phosphorus and potassium, on the other hand, the individual values of each experiment are joined by lines. The numbers of the experiments are shown on the graph. Experiments which ran concurrently under wet and dry conditions are labelled wet or dry as the case may be.

We will consider first of all the nitrogen experiments. For the first there are five values. The first four lie along a straight line, while the fifth value lies to the right of this line, indicating the intrusion of a factor limiting protein synthesis. A maximum protein value was not reached. The first three values of the second nitrogen experiment lie on the same line as the first experiment. As crystalloid nitrogen increased, a maximum protein value is reached of about 5 per cent., after which there is a fully significant drop. The third and fourth experiments were carried out under wet and dry conditions respectively. They follow a similar pattern to the first two experiments in that protein rose rapidly at first with small crystalloid changes, and then protein formation was checked and crystalloid nitrogen accumulated. In the third (wet) experiment a maximum protein value was attained but there was no decline at higher crystalloid levels, while in the fourth experiment there is no indication that a maximum had been attained. The protein level attained in the 'dry' experiment was distinctly higher than the maximum value in the 'wet' experiment.

Not only is the general pattern the same in the four experiments—a rapid rise in protein with small crystalloid changes, suddenly changing to small protein changes with a rapid rise in crystalloid nitrogen—but also in the first region (low crystalloid values) there is very little variation between the results for the four experiments. There is, however, considerable variation in the protein level attained.

We will consider next the four phosphorus experiments. Low crystalloid values correspond to high levels of phosphorus supply and high crystalloid values to low levels of phosphorus supply. Thus, the values to the right represent phosphorus deficiencies and to the left repletion. Thus phosphorus starvation has in all four experiments imposed some check on protein synthesis. Actually in the fourth experiment the protein values were slightly higher under low phosphorus supply than under high supply, but while the protein values under high supplies lie in the region of the values of the nitrogen experiments, those under low supply lie away from this region. In short, as judged by the protein/crystalloid values of the nitrogen experiments, phosphorus deficiency has imposed some restraint on protein synthesis.

The results for the potassium experiments fall into line with those for phosphorus, but the high potassium values for the second and third experiments lie some way apart from the line of the nitrogen experiments. It will be noticed that in the first experiment the protein values are higher under conditions of potassium starvation than under full potassium supply, but nevertheless, when judged in terms of crystalloid level, protein synthesis has been checked.

In both the phosphorus and potassium experiments the protein levels for the experiments carried out under dry conditions lie below those for the experiments carried out under wet conditions.

III. DISCUSSION

Petrie and Wood (1938) have published data showing the changes in total nitrogen, protein nitrogen, amide nitrogen, and amino nitrogen under varying nitrogen supply. The changes are well shown in Fig. 7 of their paper. This figure shows that as total crystalloid nitrogen increased, protein nitrogen increased to a maximum, and then as crystalloid nitrogen continued to increase, protein nitrogen diminished. This reduction in protein nitrogen is much more marked than in any of our own experiments. The same changes in protein nitrogen are apparent when either amide nitrogen or amino nitrogen are plotted against protein nitrogen. It seems clear that the crystalloid nitrogen fractions are very labile or else that they all take part in the synthesis of protein. In this connexion it is worth recalling (cf. Maskell and Mason, 1930) that a very large part (approx. 75 per cent.) of the crystalloid nitrogen in the cotton leaf may be present as residual nitrogen. When phosphorus is strongly limiting growth, as much as 25 per cent. of the crystalloid nitrogen may be present as nitrate nitrogen (cf. Phillis and Mason, 1939). How little we know of these soluble nitrogen fractions is pointed out by Vickery (1927).

As protein nitrogen reached a maximum and declined while the crystalloid fractions were increasing in concentration, it is manifest that the protein changes cannot be accounted for *solely* in terms of the crystalloid changes or in terms of the changes in any of the crystalloid fractions. At high levels of

crystalloid nitrogen some other factor intrudes itself. In Wall's (1940) experiments with potassium the same situation arises. He found that low potassium supply depressed protein nitrogen and increased amide and amino nitrogen. He thinks that potassium starvation depresses the conversion of organic crystalloid nitrogen into protein nitrogen. How potassium operates to check this conversion of crystalloid into protein nitrogen he does not indicate.

We suggested in the introduction to this paper that crystalloid nitrogen must be adsorbed before it is converted into protein. We also suggested that the surface might be enzymic. That enzymes must be taken into account is clear from Chibnall's statement that 'in the presence of adequate supplies of sugar and nitrogen—the controlling factor will be the necessary enzyme systems concerned'.

Reverting now to the changes shown in the figure. Little variation is shown at low crystalloid nitrogen levels between the results for the four nitrogen experiments. The results arrange themselves in the form of a straight line and all the values fit very closely to the line. From this we conclude that in this region crystalloid nitrogen is the only factor of any direct importance determining the protein level. In short, surface was not limiting.

As the crystalloid level continued to increase, the values depart from the straight line and divergences between the results of the four experiments appear. It will be seen that the maximum protein values in the experiments which show maxima are markedly different. We conclude that the maximum protein value is controlled by the area of the surface available for adsorption and that the area in these experiments differed. In short, the surface was limiting and may be correlated with the amount of protoplasm.

The reason for differences in the surface areas in these experiments cannot be genetic (though there must be genetic limits), for the same pure line of Sea Island cotton was used in all experiments. Environmental factors must ultimately therefore be responsible. As the surface may be under protoplasmic control, mineral factors may play a part, perhaps a dominant part. It would follow that if mineral nutrition plays a part in determining the differences in the surface areas between experiments, it might also play a part at high levels of nitrogen supply in any single experiment.

It appears, therefore, that the extent of the surface in a single nitrogen experiment may have varied at high levels of nitrogen supply as a result of a deficiency of some other element or elements, for as nitrogen supply is increased, the tissues become richer in nitrogen and poorer in potassium, phosphorus, and probably other elements. Thus, we suggest that maximum protein was determined by maximum surface and that the *decline* in protein that occurred after the maximum value was reached was due to a reduction in the surface area.¹ In short, we suggest that the maximum surface was due to

¹ The reduction in adsorption at high concentration levels characteristic of apolar adsorption may also have been a factor in causing a decline in protein.

a deficiency of some element or elements and that the reduction in the extent of the surface was due to a further deficiency of some element or possibly a lack of balance between elements.

We will now consider the results for the phosphorus and potassium experiments (see figure). We suggest that they are amenable to a similar interpretation. With the exception of the third phosphorus experiment, they show a rise followed in most experiments by a fall in protein nitrogen as crystalloid nitrogen is increased. In short, they show some resemblance to the high protein region of the nitrogen experiments, except that the decline in protein nitrogen may be more pronounced. They do not show the straight line relation shown by the nitrogen experiments at low crystalloid levels since these low levels were never attained.

Thus, it is suggested that at high levels of potassium and of phosphorus supply the protein level was determined by the crystalloid level and that at low levels the protein level was determined by the extent of the surface which in turn was determined by mineral deficiency.

Two difficulties confront this interpretation. They are shown by the potassium experiments. Firstly, at high levels of supply the values for Experiments 2 and 3 lie to the right of the nitrogen line. Some factor other than crystalloid nitrogen is evidently operating. It may have been magnesium or possibly calcium, for we know that as the concentration of potassium in the leaf rises, the concentrations of these elements decline (cf. Phillis and Mason, 1940). Secondly, at low levels of potassium supply the protein levels are very different in the three experiments. If the extent of the surface is under protoplasmic control, these differences in level might well occur as a result of deficiencies in other essential elements. Thus, in the second and third potassium experiments, the phosphorus level was much higher in the second than in the third experiment. It is therefore possible that both phosphorus and potassium were limiting the surface area in the third experiment.

We have emphasized the importance of mineral nutrition in determining the extent of the surface, but clearly if there is a protoplasmic factor many other factors will operate. Thus, we found in our second paper (1942) that the protein maximum was less in old than in young leaves. In the experiments at present under consideration we see that the 'dry' potassium and phosphorus experiments show lower protein levels than the 'wet' experiments, while in the nitrogen experiments the reverse obtains. To sum up, it is suggested that the crystalloid level controls the protein level at low crystalloid levels, while at high crystalloid levels the protein level is under the control of the surface. At intermediate crystalloid levels both crystalloid level and surface will operate. At very high crystalloid levels the adsorption of the solvent (cf. Buzagh, 1937) may depress the protein level below the maximum. It is also suggested that the surface is under protoplasmic control and consequently that any factor, including mineral supply, which affects protoplasm may also affect the surface.

Finally, a word should be said concerning Richards's (1938) work. His data include respiration rate, protein nitrogen, amino nitrogen. All are expressed on the dry weight basis. He found that respiration and protein were highly correlated and has suggested that the energy released in respiration is utilized to maintain the protein level. We have pointed out that such a correlation would be expected as both respiration and protein are associated with protoplasm. We now suggest that the area of the surface available for adsorption and not respiration is the reason for the correlation. He found apparently no unifying relation between protein and crystalloid nitrogen, but he did find treatment effects. These treatment effects were apparently due to differences in phosphorus and potassium nutrition, which we have suggested may indirectly affect the surface available for adsorption and so affect the protein level.

IV. SUMMARY

1. Four different experiments are described in which the supply of nitrogen to the roots of cotton plants was varied.

2. It was found that as the crystalloid nitrogen level increased, protein nitrogen also increased until it reached a maximum and then it declined markedly in one experiment.

3. At low levels of crystalloid nitrogen the relation between protein nitrogen and crystalloid nitrogen was much the same from one experiment to another. It was suggested that in this region the protein level is predominantly controlled by the crystalloid nitrogen.

4. At higher levels of crystalloid nitrogen the protein levels varied from one experiment to another. It was suggested that these differences in protein level were due to differences in the surface areas available for adsorption.

5. It was also suggested that in the region of high protein the extent of the surface might vary in a single experiment as a result of changes in mineral composition.

6. Phosphorus and potassium starvation caused a marked reduction in the protein level from what might be anticipated from the protein/crystalloid relation in nitrogen experiments. It was suggested that deficiencies of these elements might reduce the extent of the surface and so limit the protein level.

LITERATURE CITED

- BUZAGH, A., 1937: Colloid Systems. Technical Press Ltd., London.
CHIBNALL, A. C., 1939: Protein Metabolism in the Plant. Ox. Univ. Press, London.
HOAGLAND, D. R., 1944: Lectures on the Inorganic Nutrition of Plants. Chron. Bot. Co., Waltham, Mass., U.S.A.
MASKELL, E. J., and MASON, T. G., 1930: Movement to the Boll. *Ann. Bot.*, xliv. 657.
MASON, T. G., and PHILLIS, E., 1943: More about Nitrogen, Phosphorus, and Labile Carbohydrate. *Ann. Bot.*, N.S., vii. 399.
PETRIE, A. H. K., and WOOD, J. G., 1938: Studies on the Nitrogen Metabolism of Plants. *Ann. Bot.*, N.S., ii. 33.

344 *Mason and Phillis—Mineral Elements in the Cotton Plant. V*

- PHILLIS, E., and MASON, T. G., 1939: Preliminary Observations on Nitrogen and Phosphorus. *Ann. Bot., N.S.*, iii. 569.
- , 1940: Preliminary Observations on Potassium, Calcium, and Magnesium. *Ann. Bot., N.S.*, iv. 773.
- , 1942: Mainly Concerning Nitrogen. *Ann. Bot., N.S.*, vi. 469.
- RICHARDS, F. J., 1938: The Relation of Respiration Rate to the Carbohydrate and Nitrogen Metabolism of the Barley Leaf as Determined by Phosphorus and Potassium Supply. *Ann. Bot., N.S.*, ii. 491.
- , 1944: A Critical Examination of New Theories of the Metabolism of Major Nutritive Elements in Plants. *Ann. Bot., N.S.*, viii. 43.
- VICKERY, H. B., 1927: The Basic Nitrogen of Plant Extracts. *Plant Physiol.*, ii. 303.
- WALL, M. E., 1940: The Role of Potassium in Plants. *Soil Science*, xlix. 393.

The Effect of Ringing and of Transpiration on Mineral Uptake

A Reply to Criticism¹

BY

T. G. MASON

AND

E. PHILLIS

(Cotton Research Station, Trinidad, B.W.I.)

With one Figure in the Text

I. THE EFFECT OF RINGING

STEWART (1943) has criticized our (1940*a*) conclusion that ringing interferes with the uptake of mineral elements by the root. He claims that 'The difference as between Normal and Ringed groups is open to the interpretation that it was inherent in the plants *before they were so treated and resulted from the way in which they were grown and selected for experiment*'.

The procedure was as follows:

'Sea Island cotton plants were grown in sand cultures receiving a complete nutrient solution from which all bromine was excluded. Two plants were grown in each container. When the plants were eight weeks old 28 containers were selected in which the two plants were closely alike. The plants were then graded to give four groups each of seven containers. In each group the taller plant in the container was marked, alternately, with black or with yellow wool, and the other plant in the container with the opposite colour. Thus each group of seven containers had seven plants tagged yellow and seven tagged black. Subsequent treatment was as follows:

- | | |
|---------------|--|
| Day 0, 3 p.m. | Placed in dark room. Sand leached with water and then treated with a culture solution to which potassium bromide (2 gm./litre) was added. |
| Day 1, 8 a.m. | One group of containers collected, the seven yellow-wooled and the seven black-wooled plants forming the A and B samples respectively of the Initial collection. |
| „ 8.15 a.m. | Black-tagged plants ringed at cotyledonary node and ring covered with adhesive tape. |
| „ 8.30 a.m. | All plants brought from dark into the open. |
| „ 10.30 a.m. | Collection of three samples each of Normal (A, B, and C) and Ringed (A, B, and C) plants.' |

Thus, the plants remained for 17 hours in the dark before ringing. During this period they took up a considerable amount of bromine. At the

¹ Paper No. 36 from the Department of Physiology, Cotton Research Station, Trinidad, B.W.I.

end of 2 hours of exposure to sunlight the shoots of the Ringed and Normal plants showed no difference, but the Ringed plants had less bromine in the roots than the Normal plants. It is this difference between the bromine contents of the roots, which Steward admits to be statistically significant, that he says 'is open to the interpretation that it was inherent in the plants *before they were so treated and resulted in the way in which they were grown and selected for the experiment*'.

The method used for marking the plants in the eight samples is shown in Table I, where the taller plant in each container is shown by a capital letter and the smaller plant by a small letter. The table also shows the composition in respect of tallness of the various samples. The weights (mg.) of bromine in the roots of the various samples are also shown.

TABLE I

Container.	Group 1.		Group 2.		Group 3.		Group 4.	
1	B	y	Y	b	B	y	Y	b
2	Y	b	B	y	Y	b	B	y
3	B	y	Y	b	B	y	Y	b
4	Y	b	B	y	Y	b	B	y
5	B	y	Y	b	B	y	Y	b
6	Y	b	B	y	Y	b	B	y
7	B	y	Y	b	B	y	Y	b

B or b = Black wool; Y or y = Yellow wool.

	Initial A.	Normal A.	Normal B.	Normal C.
Yellow wool	3T+4S 122.5	4T+3S 121.9	3T+4S 129.0	4T+3S 126.5
	Initial B.	Ringed A.	Ringed B.	Ringed C.
Black wool	4T+3S 106.1	3T+4S 111.0	4T+3S 111.1	3T+4S 105.2

Steward remarks that since 'as prepared for the experiment the plants of each group fell into two batches (A and B or Normal and Ringed) which were not comparable but differed in respect of properties linked to the height of the shoot, then *one can compare the Normal plants with Initial plants A and the Ringed plants with Initial plants B*' (*italics ours*). Reference to Table I shows that there can be no justification for this procedure. It is evident that he thought that the tall plant should take up more bromine than the small plant. This was clearly not the case, for the Initial A sample contained only three tall plants and yet exceeded the B sample, with its four tall plants, in bromine content. For Group 3 also the small (Normal) sample exceeded the tall (Ringed) sample in bromine content.¹ It is also evident that he did not understand the method used for marking the plants in the eight samples; he failed apparently to note that in 'one group of containers the seven yellow-wooled and the seven black-wooled plants (formed) the A and B samples

¹ Sum of four 3T+4S samples = 467.7 mg. bromine.

„ „ 4T+3S „ = 465.6 „ „

respectively of the Initial collection'. Thus his contention that 'The difference as between Normal and Ringed groups is open to the interpretation that it was inherent in the plants *before they were so treated and resulted from the way in which they were grown and selected for experiment*' cannot be accepted.

Though the mean difference between the bromine contents of the Ringed and Normal roots was statistically significant, it is nevertheless true, as Steward points out, that this difference is no greater than that which already existed between the duplicate Initial samples. We accordingly present the results of another experiment dealing with the effect of ringling on mineral uptake by the root.

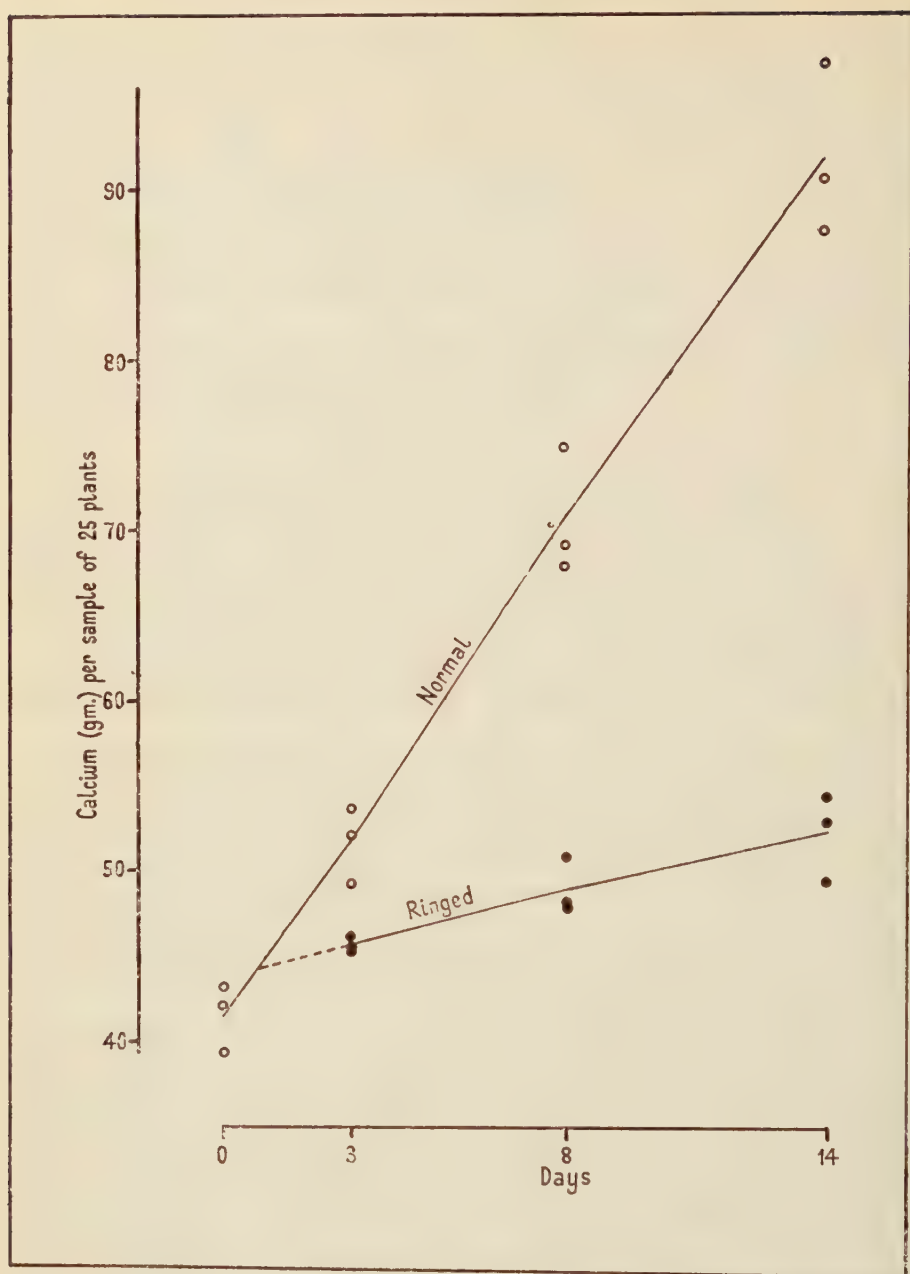
We (1940) have already described this experiment in connexion with the effect of ringling on upward movement of solutes, but there was one group of plants we did not mention in this earlier paper. Briefly, the procedure was as follows: Plants were grown in the field and consisted of a short main stem and two branches of approximately equal size. The plants were 15 weeks old when the experiment began. There were three groups of plants. In one neither branch was ringed (Normal); in the second one branch was ringed; and in the third both branches were ringed at their bases where they joined the main stem. There were an Initial and three collections during a period of 14 days. The results for the Normal and 1-Ring groups have already been presented (1940*a*). We now present the results for calcium¹ uptake by the whole plant in the 2-Ring group and compare it with uptake in the Normal group.

The figure shows the results expressed per sample of 25 plants. There were 3 samples at each collection, and they are shown individually in the figure. It will be clear that ringling has greatly interfered with the uptake of calcium and that the check is already well marked at the first collection on day 3. The uptake between days 3 and 14 was approximately linear. If the line representing uptake by the Ringed plants between days 3 and 8 is produced towards day 0, it meets the line representing uptake by the Normal group at a point approximately 20 hours from the Initial collection. Thus ringling must have interfered with uptake within this period.

The Initial collection was made about 9 a.m., but as ringling was done after this, and as there were approximately 700 rings to cut, this operation was not completed till much later. The interval between the mean time of ringling and the first collection of ringed plants was less by 4-5 hours than the interval between the Initial collection and the first collection of Normal plants. Thus uptake by the Ringed plants must have been checked in $20 - 4\frac{1}{2} = 15\frac{1}{2}$ hours. Furthermore, the check in calcium uptake could not have taken place suddenly. It must have taken effect in less than $15\frac{1}{2}$ hours.

However, even when every allowance is made, it is possible that uptake by the root was checked in a shorter period in the bromine experiment criticized by Steward than in the present experiment. As the plants in the bromine

¹ Results are also available for nitrogen, phosphorus, magnesium, and chlorine. As they are essentially similar to those for calcium, they are not presented.



Calcium Uptake in Normal and in Ringed Plants.

experiment were placed in the dark for 17 hours before ringing, their roots would have contained very little carbohydrate. If carbohydrate starvation in the roots of the Ringed plants is the reason for the check in mineral uptake, a rapid response in the Normal plants would be expected on exposure to light. In the present experiment, on the other hand, the roots of the Ringed plants would not have suffered to the same extent from carbohydrate starvation, for the plants were exposed to daylight for several hours immediately before ringing. A slower response might therefore be anticipated.

Finally, a few words should be said concerning the possibility that ringing checks mineral uptake as a result of a check in transpiration and not in transport. In our paper we suggested that a check in transpiration might check mineral uptake by increasing the concentration in the root or by diminishing the supply of oxygen carried to the root or by both. In the *bromine* experiment the amounts of bromine were the same in the tops of the Ringed and Normal plants. Moreover, the amount of bromine was less in the roots of the Ringed than in the roots of the Normal plants. Thus in this experiment there is no suggestion that bromine uptake was checked as a result of a check in transpiration. In the *calcium* experiment 92 per cent. in the Normal and 87 per cent. in the Ringed plants of the total calcium absorbed in the first 3 days was in the tops, while the increase in 3 days in the calcium content of the region below the ring in the Ringed plants was only half of that in the roots of the Normal plants. Admittedly the amount of calcium in the region below the ring may not be a reliable guide to the concentration in the absorbing region of the root. On the other hand, the difference in the amounts was so great that it is unlikely that the concentration of calcium in the absorbing region of the root can have been greater in the Ringed than in the Normal plants. At later collections there was an actual loss of calcium from the roots of the Ringed plants along with a gain in the tops. It seems very unlikely that the check in uptake can have been caused by a check in transpiration.

II. THE EFFECT OF TRANSPIRATION

In a second experiment in the same paper (1940*a*) we concluded that 'Increased transpiration caused increased uptake of bromine in ringed plants'. We did not conclude, as Steward says, 'that increased transpiration increased the uptake of bromine by cotton plants'. The essential feature of the experiment was ringing. The plants were ringed in order to eliminate any factor associated with the export of metabolites from the leaf to the root. Ringed plants exposed to sunlight were compared with ringed plants kept in darkness. It was found that the plants exposed to sunlight absorbed much more bromine than the plants kept in the dark.

The difference in uptake is not disputed by Steward, but our interpretation that transpiration was responsible is disputed. Thus he says: 'There is every reason to believe that, of all the varied effects which may accrue from placing a shoot in the light (high concentration of respiratory substrates, oxygen-carbon

dioxide relations favourable to respiration, greater transpiration, increased enlargement of laminae &c.), it is the effect of light on growth of the shoot which is the operative one and which determines the salt uptake of plants which, previous to experiment, were exposed to full salt supply.' Thus he thinks the real difference in uptake was in some way due to a difference in the development of the *shoots* of the exposed and dark plants rather than to a difference in transpiration.

Of the varied effects which may accrue from placing a shoot in light the first he mentions is 'high concentration of respiratory substrates'. Now Mason and Maskell (1928) have shown that ringing stops the transport of sugar to the root, so unless he had in mind some respiratory substrate other than sugar, and one moreover which travelled downwards in the wood, his suggestion is meaningless.

The second effect he mentions is 'oxygen-carbon dioxide relations favourable to respiration'. He presumably was thinking of the effects of carbon assimilation on the oxygen-carbon dioxide relations in the leaves of plants exposed to sunlight. We must ask how is oxygen transmitted to the root in ringed plants? It could only travel *downwards* in the wood (cf. Mason and Phillis, 1936).

The third effect mentioned is 'transpiration' and the fourth 'increased enlargement of the laminae'. How, it may be asked, can the leaf area affect the root in a ringed plant except by transpiration? It seems clear that apart from transpiration the only other possible way in which the shoot can affect the root of a ringed plant is by means of some factor transmitted downwards through the wood. In the previous section we have shown that some factor is transmitted from the shoot to the root which affects mineral uptake, but the transmission of this factor is stopped by ringing.

In our paper we suggested that transpiration might have affected bromine uptake by lowering the concentration in the root. As Steward points out, the total amount of bromine (average) was the same in the roots of the Dark and Open plants. Strange to say, he did not notice that in the roots of the exposed plants the weight of bromine ranged from 36.4 to 49.1 mg. per sample. The sampling is obviously such that no conclusion can be drawn concerning a difference in bromine concentration in the roots of the Dark and Open plants. Moreover, there might well be a *reduced* concentration in the absorbing regions of the root and an *increased* concentration in the older parts of the root, just as in the tops in response to increased transpiration, with the net result that there might be no change in the root as a whole.

Other points criticized by Steward we will not reply to as they do not affect our conclusion that 'increased transpiration caused increased uptake of bromine in ringed plants'.

There is one remarkable feature of Steward's criticism of *this* experiment; he does not in a single instance mention that both the *exposed* and *dark* plants were ringed between the foliage region and the root, yet our statement that 'all plants were ringed at (the) cotyledonary node' seems perfectly clear. To

conclude, his criticism would have been valid if the plants had not been ringed, but as they *were* ringed it is irrelevant.

III. SUMMARY

1. Criticisms by Steward of the interpretation of the results of experiments on the effects of ringing and of transpiration on mineral uptake by the cotton plant are discussed.

2. It is shown that the main criticism of the *ringing* experiment is based on a misunderstanding of the actual experimental procedure employed.

3. Results for another ringing experiment are presented which support our conclusion that ringing rapidly interferes with the uptake of solutes by the roots.

4. His criticism of our conclusion that increased transpiration caused increased uptake in ringed plants is shown to be irrelevant, for while his criticism would be valid for normal plants, it is not applicable to plants ringed between the foliage region and the root.

LITERATURE CITED

- MASON, T. G., and MASKELL, E. J., 1928: A Study of Diurnal Variation in the Carbohydrates of Leaf, Bark and Wood, and of the Effects of Ringing. *Ann. Bot.*, xlii. 1.
— and PHILLIS, E., 1936: Oxygen Supply and the Activation of Diffusion. *Ibid.*, cxcix. 455.
PHILLIS, E., and MASON, T. G., 1940: The Effect of Ringing on the Upward Movement of Solutes from the Root. *Ibid.*, N.S., iv. 635.
— — 1940a: The Effect of Ringing and of Transpiration on Mineral Uptake. *Ibid.*, N.S., iv. 645.
STEWART, F. C., 1943: The Effect of Ringing and Transpiration on Mineral Uptake. *Ibid.*, N.S., vii. 89.

The Effect of Extreme Desiccation on the Viability of Cotton Seed¹

BY

E. PHILLIS

AND

T. G. MASON

(*Cotton Research Station, Trinidad, B.W.I.*)

With four Figures in the Text

I. INTRODUCTION

CHRISTIDIS (1940) found that the longevity of cotton seed was dependent on its moisture content. High moisture content was associated with short life and low resistance to high temperature. He was concerned only with comparatively wet seeds (minimum moisture content of 9 per cent.). Simpson (1942) has also investigated the effects of humidity and temperature on the longevity of cotton seed. He found that 'If the moisture content is low' (7 per cent.) 'cotton seeds can withstand high temperatures without rapid deterioration, and if the temperature is kept low, they are tolerant of high moisture, but both temperature and humidity cannot be high if rapid deterioration is to be prevented'. A survey of the literature suggests that the drier, the better for cotton-seed storage. The present paper is concerned with an investigation of this suggestion.

II. PROCEDURE

Freshly harvested Sea Island cotton seeds were thoroughly air dried (moisture content 7.7 per cent.). Samples of approximately 7,000 seeds were then placed in desiccators containing mixtures of sulphuric acid and water to give humidities varying from 0 to 80 per cent. of that of water. At intervals the acid was changed so that the relative humidity in each desiccator kept constant throughout the duration of the experiment. Samples of seed were withdrawn at intervals, delinted with sulphuric acid, washed with water, and planted in sand for viability tests. The sand was kept in a large flat tray approximately 4 in. deep, with free drainage, and was kept moist by frequent watering. The seeds (in duplicate samples of 100) were planted 1 in. apart

¹ Paper No. 37 from the Physiological Department of the Cotton Research Station, Trinidad, B.W.I.

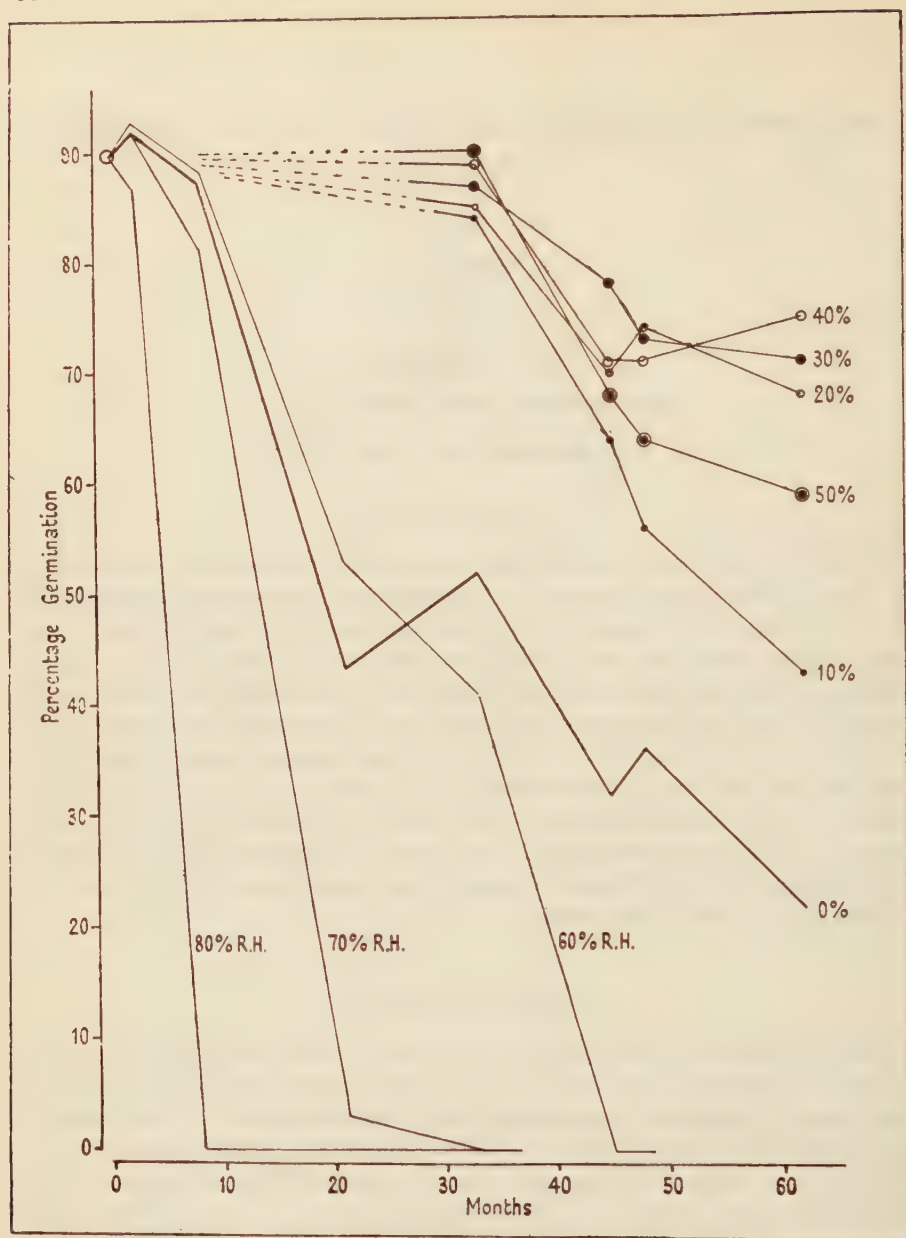


FIG. 1. The relation between germination and time of storage for seeds kept at various relative humidities expressed as percentages.

and $\frac{1}{2}$ in. deep. The tray was covered until the seeds began to emerge above the surface. The temperature was not controlled either during storage or germination, but ranged about 75–85° F. Only seeds which actually emerged through the sand were counted as germinated. The more usual procedure is

to count emergence of the radicle as germination, but we found that dried seeds might get so far and that then they invariably died off.

III. RESULTS

The results are shown in Fig. 1. The percentage of seeds germinated has been plotted against time expressed in months. The sampling is not very good,

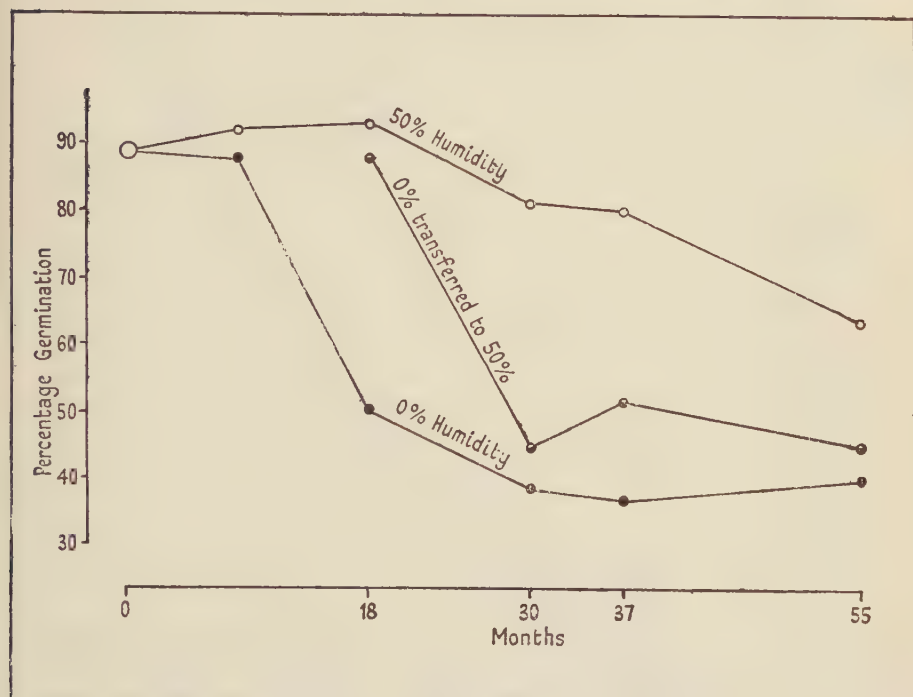


FIG. 2. The effect on germination of transferring seeds from 0 per cent. relative humidity to 50 per cent. relative humidity for 6 months.

but the general trend of events is quite clear. The seeds in the high humidity chambers showed rapid deterioration. The 80 per cent. treatment had completely lost the power of germination within a period of 8 months. They were followed by the 70 per cent. treatment (33 months) and the 60 per cent. (40 months).

Main interest is, however, centred on the behaviour of the low humidity treatments. Thus the 0 per cent. treatment dropped in the course of 5 years from 90 per cent. to 22 per cent. germination. It was followed by the 10 per cent. treatment which dropped to 43 per cent. and then the 50 per cent. which declined to 59 per cent. There was very little difference between the behaviours of the 20, 30, and 40 per cent. treatments. After 5 years they declined to about 70 per cent. The outstanding feature of the results is the surprising loss of

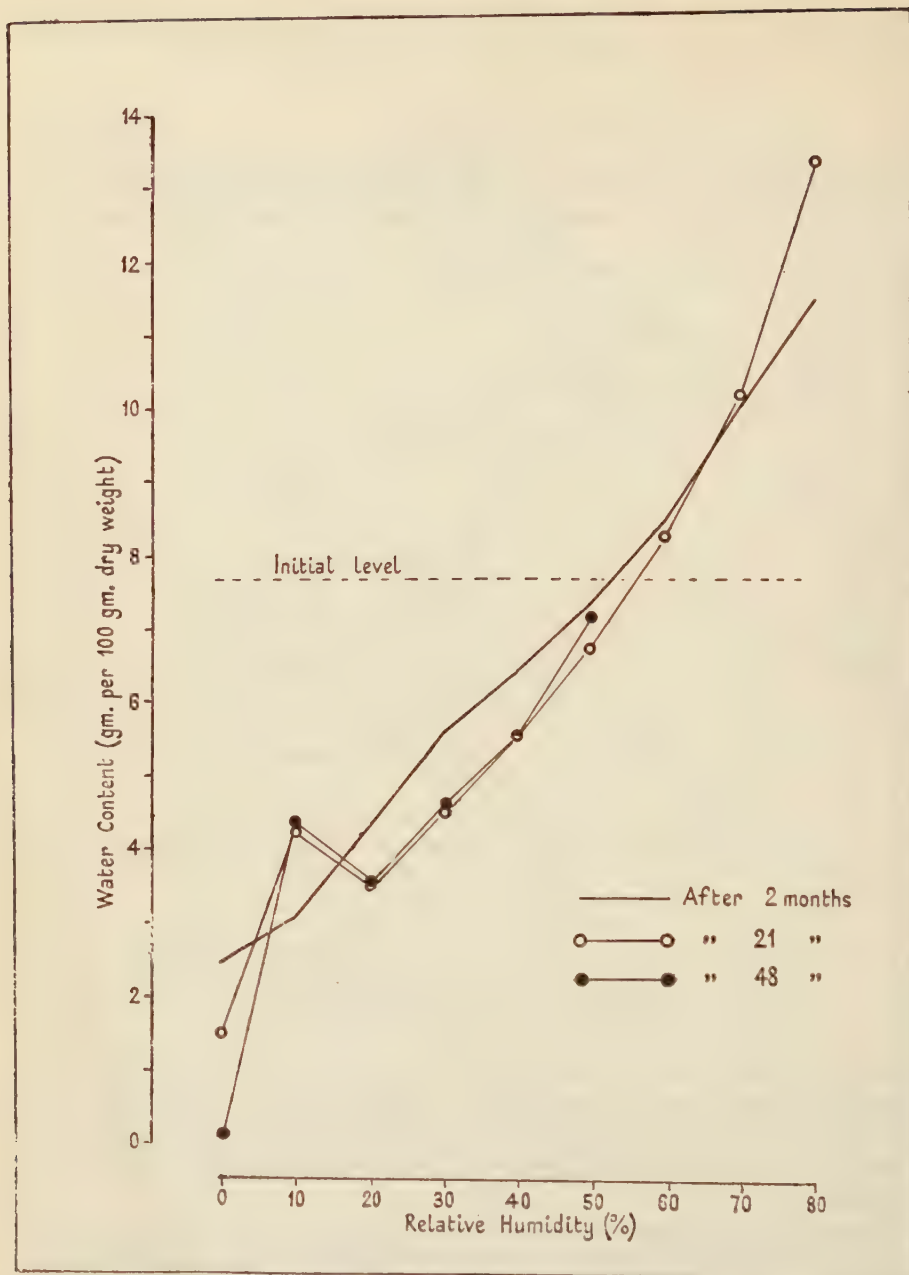


FIG. 3. The moisture content of the seeds (water (gm.) per 100 gm. dry weight) after 2, 21, and 48 months storage at various relative humidities.

germinating power of the seeds maintained under conditions of very high aridity.

We thought that these seeds might increase their germinating capacity if

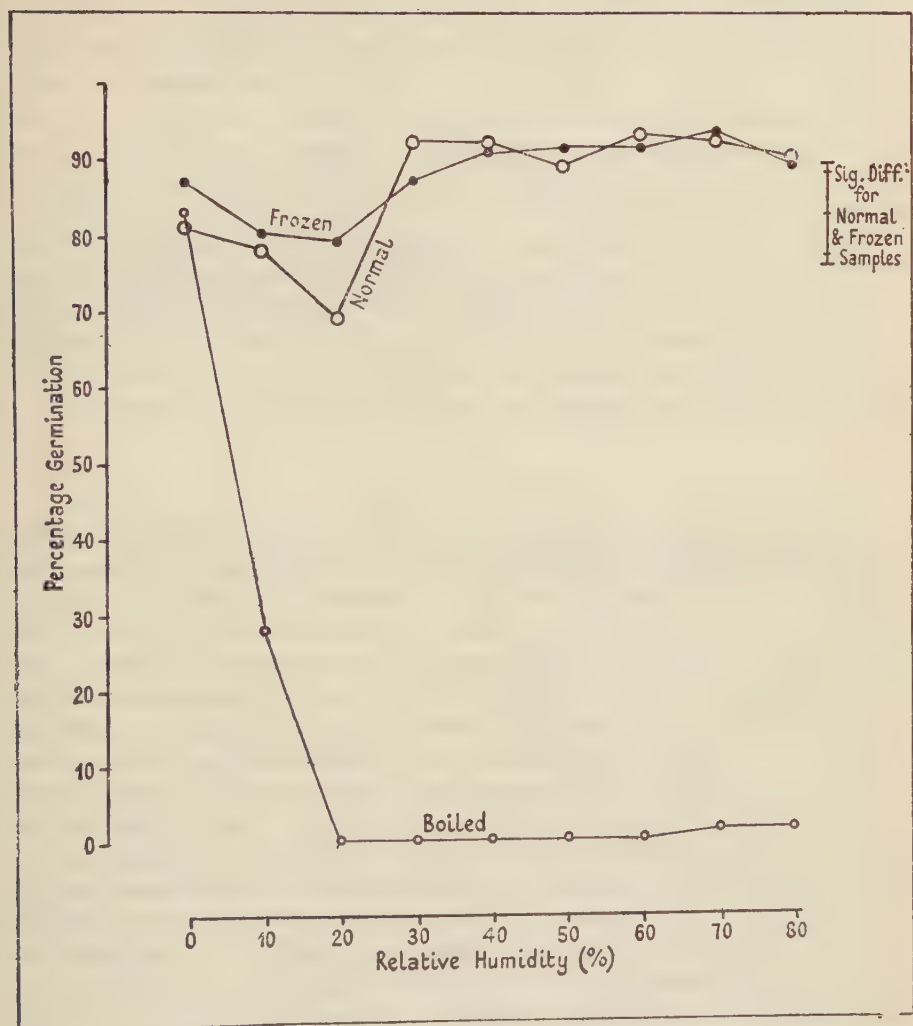


FIG. 4. The effects of heat and cold on germination of seed stored for 2 months at varying relative humidities.

they were transferred to a higher humidity *before* being placed in the sand for the germination test. Seeds from the 0 per cent. treatment were accordingly transferred from the 0 to a 50 per cent. chamber. They were left for a period of 6 months and then compared with the 0 and 50 per cent. treatments. This was done on four occasions. The results are shown in Fig. 2. It will be seen that they did to some extent increase their power of germination. The trial

made after 18 months showed nearly the same percentage-germination as the seeds that had been kept continuously at 50 per cent. The restoration of germinating power was less at subsequent trials.

The moisture content of the seeds (gm. water per 100 gm. dry weight) was determined after 2, 21, and 48 months and is shown in Fig. 3. The values for the 10 per cent. treatment for 21 and 48 months are anomalous. If we disregard these, it would appear that for treatments less than 60 per cent., moisture equilibrium was not attained in 2 months. There is also a suggestion that it was not attained in the 0 per cent. treatment even after 21 months. It would appear that at very low humidities, moisture equilibrium may be attained with great slowness.

After seed had been stored for 2 months we put small samples of all the treatments into $\frac{1}{2}$ in. Pyrex test-tubes. One set was then immersed in boiling water for half-an-hour, while another set was put into a large thermos jar containing solid carbon dioxide and left overnight. Next day these samples were sown along with a control set. Germination results are shown in Fig. 4. Cold had no effect on germination, but heat killed all but the driest seeds.

IV. DISCUSSION

The most interesting results of this investigation are the depressing effect of intensive drying on the germinating power of cotton seed, and the reduction of such effect when the seeds are '*conditioned*' by placing in a less arid atmosphere for 6 months before sowing. We are unable to continue this work because the Cotton Research Station is closing down, but obvious lines of continuance are (1) the effects of conditioning in humidities other than 50 per cent., and (2) the effects of the duration of conditioning on the germinating power. The dried seeds swelled readily when wetted and in some cases the radicle emerged a few millimetres, but this was invariably followed by the death of the seed. It is possible that water uptake by very dry seeds is so rapid that it kills the seeds through structural deformation in the same manner as the formation of ice crystals or too rapid recovery from plasmolysis kills plants. Iljin (1934) remarks: 'In the process of thawing the protoplast rapidly imbibes water, is violently dilated and perishes in consequence. If this dilation is detained or hindered by the addition of a sugar solution previously to thawing, the tissue survives.'

V. SUMMARY

1. Cotton seeds stored under conditions of very high aridity do not maintain their germinating capacity as well as seeds stored at medium humidities.

2. When seeds under such dry conditions are exposed for 6 months to medium humidities before testing, i.e. are conditioned, they exhibit an appreciably higher percentage germination than similar seeds not conditioned.

3. It is suggested that with very dry seeds the first stages of re-moistening must be carried out slowly if death of the seed is to be avoided.

4. The optimum relative humidity for storing cotton seed at temperatures between 75 and 85° F. would appear to be about 30 per cent. If satisfactory processes can be devised for conditioning stored seeds before germination, it may be that storage under as dry conditions as possible would be the best.

LITERATURE CITED

- CHRISTIDIS, B. G., 1940: The Viability of Cotton Seed as Affected by its Moisture-Content. *Emp. Journ. Exp. Agric.*, viii. 148.
- ILJIN, W. S., 1934: The Point of Death of Plants at Low Temperatures. *Bull. de l'assoc. Russe pour les Recherches Scient. à Prague*, i. 135.
- SIMPSON, D. M., 1942: Factors Affecting the Longevity of Cotton Seed. *Journ. Agric. Res.*, lxiv. 407.

The Seed Hairs of *Gossypium*

BY

J. B. HUTCHINSON AND S. G. STEPHENS

(*Cotton Research Station, Trinidad*)

AND

K. S. DODDS

(*Imperial College of Tropical Agriculture, Trinidad*)

With Plate IV

IT is characteristic of the genus *Gossypium* that all species have unicellular hairs growing from the seed coat. They vary in quantity from a very fine coat scarcely visible to the naked eye to the copious coating of long hairs characteristic of the true cottons. It has been shown elsewhere (Hutchinson and Stephens, 1944) that these seed hairs are of two main types. Those borne on the seeds of the wild diploid species are simple, unconvoluted hairs, whereas those borne by the cottons of the Old and New Worlds are flat, spirally twisted (convoluted) ribbons. It is the latter which constitute the cotton lint of commerce. Additional observations have recently been made on the simple hairs of the wild diploid species which throw light on the origin of the New World cottons. The $n = 26$ chromosome cottons of the New World are generally accepted as having arisen by amphidiploidy within the genus *Gossypium* following hybridization between an Old World diploid cotton and a wild American diploid lintless species. Stephens (1944) has given phenogenetic evidence in support of the view that the American parent must have been one with entire leaves, and it will be shown here that the choice may be further limited, since not all the American wild species have seed hairs suitable for forming lint in combination with the lint hairs of the Old World cottons.

In all seed hairs in *Gossypium* growth in length is followed by secondary thickening, which takes place by the deposition of cellulose within the hair cuticle. The amount of this secondary thickening varies from a comparatively thin layer, as in all cottons, to a deposit which occupies practically the whole volume of the cell. The wild diploid species examined can be grouped according to the extent of this secondary thickening.

Three species having a rather copious fuzzy covering on the seed, *G. stocksii* from India, *G. anomalum* from Africa, and *G. raimondii* from Peru, have seed hairs that are very much alike. They are smooth and straight in outline and fairly uniformly thickened, leaving a continuous lumen occupying rather less than one-third of the diameter of the hair. In hairs rather less

thickened than normal, occasional convolutions can be observed. A section of such a hair from *G. raimondii* showing a typical convolution is illustrated in Pl. IV, Fig. 1.

The Mexican species *G. aridum* also has a rather copious fuzzy coating on the seeds. The hairs are smooth and straight. The degree of secondary thickening is very variable, hairs in which the lumen accounts for two-thirds of the hair diameter being common. Nevertheless, though incompletely thickened hairs are common they rarely show convolutions.

The remaining three American species examined, *G. thurberi*, *G. armourianum*, and *G. klotzschianum* var. *davidsonii*, have only extremely reduced seed hairs. Those of *G. armourianum* are very closely appressed to the seed. Those of the other two are so scanty and so short that to the naked eye the seeds appear almost glabrous. In all three the hairs are irregular in outline, often wavy or even kinked, and not infrequently variable in diameter along their length. Those of *G. armourianum* and *G. klotzschianum* var. *davidsonii* are coarse, usually heavily thickened, and bear no convolutions even when incompletely thickened. Secondary thickening in hairs of *G. thurberi* is irregular, so that in some places the lumen is practically obliterated, and in others is fairly large, giving it a beaded appearance. No convolutions were seen. A section of a hair of *G. thurberi* is illustrated in Pl. IV, Fig. 2.

The most complete thickening observed was in the Australian *G. sturtii*. The hairs are short and inconspicuous. They are smooth and straight in outline, and secondary thickening is practically complete, the lumen only being visible as a fine line down the centre.

As stated earlier, on the generally accepted hypothesis of the origin of the New World ($n = 26$) cottons, it is to be expected that the 'seed hair producing mechanisms' of the two diploid parents would be so balanced as to be capable of lint production in the amphidiploid, since all known natural amphidiploids, whether cultivated or not, bear true lint. The nature of the seed hairs borne by colchicine synthesized allopolyploids is therefore of considerable significance in determining the probable ancestry of the New World cottons, and the data available will now be reviewed.

G. thurberi has been successfully crossed with Old World cottons by several workers, and the behaviour of allopolyploids induced by treating the hybrids with colchicine has been reported by Harland (1940) and Beasley (1942). An allopolyploid was obtained at the Cotton Research Station (C.R.S.) by treating a *G. arboreum* \times *G. thurberi* hybrid with colchicine, and flowers were pollinated with pollen of New World cottons. Seeds have recently been obtained and their hairs examined. Through the kindness of Dr. M. Brown we have also been able to examine the hairs on seeds produced by pollinating (with New World pollen) Dr. J. O. Beasley's hexaploid 2 (*G. hirsutum* \times *G. thurberi*) and a tetraploid [$2 (G. arboreum \times G. thurberi)$] \times *G. hirsutum*. Since the seed coat is a maternal tissue, the hairs examined were in all cases from non-segregating generations, and therefore carried whole chromosome sets from their respective parents. It will be convenient to designate the various

chromosome sets involved in these polyploids by the system of nomenclature proposed by Beasley (1942). He assigned the letter A to the set carried by the Old World diploid cottons, and D to that of the American diploid wild species. The complement of the New World allopolyploid cottons he termed (AD). The complements of different species of the same group were distinguished by numerical subscripts. Then the sets concerned in the polyploids under discussion are as follows:

<i>G. arboreum</i>	A ₂
<i>G. thurberi</i>	D ₁
<i>G. hirsutum</i>	(AD) ₁

and the hybrid material was constituted as follows:

Plant.	Parentage.	Chromosome complement.
C.R.S. tetraploid	2 (<i>G. arboreum</i> × <i>G. thurberi</i>)	2A ₂ 2D ₁
J. O. B.'s hexaploid	2 (<i>G. hirsutum</i> × <i>G. thurberi</i>)	2(AD) ₁ 2D ₁
J. O. B.'s tetraploid × <i>G. hirsutum</i>	[2 (<i>G. arboreum</i> × <i>G. thurberi</i>)] × <i>G. hirsutum</i>	A ₂ D ₁ (AD) ₁

Since there appear to be no differences in the type of hair developed by different A sets in diploid Old World cottons, the possibility that the A set in *G. hirsutum* differs markedly from that in *G. arboreum* need not be considered. Large differences in the type of hair carried by species with different D sets have been demonstrated above, and in considering the hair structure in these polyploids, the three following possibilities must be taken into account: (1) That the D chromosome set in the New World species is of the same value as the A set in lint formation; (2) that the D set in the New World species is inert in lint formation; (3) that the D set in New World cottons is equivalent to the D₁ set of *G. thurberi*.

The proportion of lint inducing to *G. thurberi* chromosomes on these three possibilities will be as follows:

	D from N.W. equals A.	D from N.W. is inert.	D from N.W. equals D ₁ .
2A ₂ 2D ₁ .	1 : 1	1 : 1	1 : 1
2 (AD) ₁ 2D ₁ .	2 : 1	1 : 1	1 : 2
A ₂ D ₁ (AD) ₁ .	3 : 1	2 : 1	1 : 1

On examination it was found that the seed hairs of the C.R.S. tetraploid 2A₂ 2D₁ and Dr. Beasley's hexaploid 2(AD)₁ 2D₁ were very closely alike. They were wavy in outline and the lumen was irregular in diameter. Convolutions only occurred very rarely, though they were slightly more frequent in the hexaploid than in the tetraploid. A section of a hair of the tetraploid 2A₂ 2D₁ is illustrated in Pl. IV, Fig. 3. A typical convoluted cotton hair is illustrated in Pl. IV, Fig. 4 for comparison. The hairs of Dr. Beasley's tetraploid × *G. hirsutum*, A₂ D₁ (AD)₁ were much more cotton-like. They were smooth and straight, and bore frequent convolutions.

Evidently the type of seed-hair development and the formation of convolutions

in the polyploids are as would be expected if the D set in the New World cottons is inert as regards these two processes. In hair quantity, on the other hand, seeds of the C.R.S. tetraploid $2A_2 2D_1$ were almost naked. Those of Dr. Beasley's tetraploid $A_2 D_1 (AD)_1$ had a copious covering, and the hexaploid $2(AD)_1 2D_1$ was intermediate. In this respect the D set in the New World cottons is of similar value to the A set, as might be expected if it was derived from a species with a copious fuzz.

Attempts to obtain amphidiploids from Old World cotton $\times G. raimondii$ F_1 s have not yet succeeded, but an amphidiploid has been obtained from a cross of *G. arboreum* $\times G. anomalum$ (Hutchinson and Stephens, 1944), and the seed hairs on this type may be regarded as representative of those obtainable by crossing Old World cottons with fuzzy-seeded wild species. The seeds bore a copious covering of hairs, which were smooth in outline, regularly thickened, and frequently convoluted. Of all the seed hairs examined they were morphologically most closely similar to true lint.

It may be concluded that the only amphidiploids likely to give true lint hairs on the seed are those involving an Old World cotton and a fuzzy-seeded wild species with smooth, regularly thickened seed hairs bearing occasional convolutions. Among the wild species at present known, *G. stocksii*, *G. anomalum*, and *G. raimondii* meet these requirements. Only the *G. arboreum* $\times G. anomalum$ polyploid has so far been synthesized. Though 40–50 per cent. female fertile, it is almost male sterile.

The conclusion that a linted allopolyploid could be derived from a cross of *G. stocksii* with an Old World cotton raises questions of considerable theoretical importance. *G. stocksii* is indigenous in Sind, the most ancient area of cultivation of the Old World (A-bearing) cottons (Gulati and Turner, 1928). By whatever means the Pacific gap was bridged when the A- and D-bearing species were brought together and the New World allopolyploids formed, it can scarcely be doubted that the A-bearing species and *G. stocksii* were in proximity at an earlier date, and they have inhabited contiguous areas continuously up to the present day. Hybridization is so easy that natural hybrids must have arisen frequently, and the chromosomes of *G. stocksii* (Beasley's E complement) are widely differentiated from the A set (Skovsted, 1937), so that good pairing might be expected if an allopolyploid arose. Yet, in spite of greater opportunities than were available for the formation of the New World cottons, no allopolyploid involving the A and E complements is known, and it may be concluded that other requirements must be met besides propinquity, cross compatibility, and chromosome differentiation.

The general association of vigour with successful polyploidy has led to the assumption that one of the advantages of polyploidy is the means it provides of fixing heterosis. To quote Huskins and Smith (1934): 'Hybrid polyploids have in their immediate diploid ancestor hybrid vigour which is presumably due chiefly to the interaction of a number of such highly differentiated allelomorphs. By chromosome doubling these become polymeric genes. An autogamous allopolyploid should retain the specific type of hybrid vigour of its

diploid ancestor.' It seems to have been overlooked in discussions of polyploidy that not all hybrids between species with differentiated chromosome complements exhibit heterosis. In *Gossypium* a wide range in vigour is observable in diploid species hybrids. Hybrids available for study at the Cotton Research Station may be grouped as follows:

Cross.	Chromosome complements. ¹	Fertility.
A. Vigour equal to, or rather better than, the diploid parents		
<i>G. arboreum</i> × <i>G. herbaceum</i>	A ₂ × A ₁	Fertile
<i>G. arboreum</i> × <i>G. anomalum</i>	A ₂ × B ₁	Slightly fertile
<i>G. herbaceum</i> × <i>G. anomalum</i>	A ₁ × B ₁	Slightly fertile
<i>G. arboreum</i> × <i>G. raimondii</i>	A ₂ × D ₅	Sterile
<i>G. herbaceum</i> × <i>G. raimondii</i>	A ₁ × D ₅	Sterile
<i>G. anomalum</i> × <i>G. davidsonii</i>	B ₁ × D ₃	Sterile
<i>G. harknessii</i> × <i>G. armourianum</i>	D ₂ × D ₂	Fertile
B. Vigour fair but below that of the parents		
<i>G. arboreum</i> × <i>G. thurberi</i>	A ₂ × D ₁	Sterile
<i>G. aridum</i> × <i>G. thurberi</i>	D ₄ × D ₁	Very slightly fertile
C. Vigour poor		
<i>G. arboreum</i> × <i>G. aridum</i>	A ₂ × D ₄	Sterile
<i>G. herbaceum</i> × <i>G. aridum</i>	A ₁ × D ₄	Sterile
<i>G. arboreum</i> × <i>G. stocksii</i>	A ₂ × E ₁	Sterile
<i>G. herbaceum</i> × <i>G. stocksii</i>	A ₁ × E ₁	Sterile
D. Hybrids weak and short-lived		
<i>G. anomalum</i> × <i>G. stocksii</i>	B ₁ × E ₁	Dies before flowering

It will be seen that both the fully fertile crosses and both the slightly fertile crosses occur in the class containing the most vigorous hybrids, and it is evident that the value of this class as a source of good allopolyploids is very much reduced by the fact that over half the crosses involve chromosome complements between which homology is high. The other three classes, on the other hand, include hybrids that would have little chance of survival to maturity in competition with the parent species. Even if chromosome doubling occurred before they were extinguished the resulting allopolyploid would not survive unless doubling resulted in a great improvement in vigour. Of this there appears to be no evidence. Two of the vigorous hybrids, *G. arboreum* × *G. anomalum* and *G. anomalum* × *G. davidsonii*, have been doubled. Their polyploid derivatives are in general rather stouter in the stem, more compact, and have rather larger and thicker leaves, but they differ little from the corresponding diploids in general vigour. One of the fairly vigorous hybrids, *G. arboreum* × *G. thurberi*, has also been doubled, and the only visible difference between the diploid and the polyploid is that the polyploid is female fertile, whereas the diploid is completely sterile.

A fourth requirement may now be added to those given above as prerequisite for successful allopolyploidy. The diploid hybrid must be at least as vigorous as the parental species. There is a marked tendency among the

¹ Beasley's (1942) nomenclature.

hybrids listed above for loss of vigour to accompany increasing chromosome differentiation. Apparently it is only where chromosomal differentiation has gone on without any great loss of hybrid vigour that a successful allopolyploid may arise, and it must be concluded that the allopolyploids established in nature represent a very small and very rigorously selected sample of those that theoretically might have arisen. The absence of allopolyploids carrying the A and E complements is now explicable. Old World cotton \times *G. stocksii* hybrids vary considerably in vigour. Some are fair and others very poor, but all are inferior to the parental species, and would therefore not be expected to give rise to successful allopolyploids.

The origin of the D set in the natural allopolyploid (AD) cottons of the New World may now be considered. Relatives of *G. aridum* and *G. thurberi* can be ruled out as ancestral types both on account of the low vigour of hybrids of these species with Old World cottons, and because their seed hairs are not of the type that gives convoluted lint hairs with the lint-inducing genotype of the Old World cottons. Relatives of *G. harknessii*, *G. armourianum*, and *G. klotzschianum* and its var. *daviesii*, which have not been successfully crossed with Old World cottons, can also be eliminated on the unsuitability of their seed hairs. There remains *G. raimondii*, which, though it can only be crossed with difficulty with Old World cottons, gives a vigorous diploid hybrid. Phenogenetic evidence on leaf-shape development has already been presented (Stephens, 1944) in support of the view that this hybrid is similar in behaviour to the New World cottons. In size, plant habit, shape and number of bract teeth, and in flower characters it resembles the New World cottons, and moreover the genotype of the *G. raimondii* parent in regard to seed hairs is such as to give convoluted lint hairs with the chromosome complement of the Old World cottons. It may therefore be concluded that the D complement of the New World cottons was derived from an American wild species closely related to *G. raimondii*, and considerably differentiated from all other American wild species that have been studied.

SUMMARY

The seeds of all species of *Gossypium* bear hairs. These are of two main types: the flattened, convoluted hairs of the cultivated cottons and the simple, untwisted hairs borne by the wild species. The wild species may be subdivided according to the quantity and kind of the simple hairs.

The variation in the type of seed hair observed among American wild species is of importance for the determination of the relationships of the American species involved in the hybrid from which the New World allopolyploid cottons sprang. From a comparison of the seed hairs of polyploids involving *G. thurberi* and various true cottons it is shown that the D genome (Beasley's nomenclature) carried by the allopolyploids is inert in the formation of convoluted lint hairs, but has an important influence on the quantity

of seed hairs developed. It is concluded that the only known D-carrying wild species that would behave in this way in an allopolyploid with an Old World cotton is *G. raimondii*, the suitability of which has already been noted in another connexion by Stephens (1944).

G. stocksii has the same type of seed hair as *G. raimondii*, and has been in contact with Old World cottons for a very long period. Possible reasons why no allopolyploids have arisen from crosses between Old World cottons and *G. stocksii* are discussed. It is concluded that in general genom differentiation leads to low vigour in hybrids, and it is only in a comparatively narrow range where sufficient chromosome differentiation is combined with good vigour in the F_1 hybrid that there is any prospect of successful allopolyploidy.

We are indebted to Dr. M. S. Brown, Texas Agricultural Experiment Station, for seeds of the late Dr. Beasley's polyploids. The figures are by Mr. F. Whitburn.

LITERATURE CITED

- BEASLEY, J. O., 1942: Meiotic Chromosome Behaviour in Species Hybrids, Haploids and induced Polyploids in *Gossypium*. *Genetics*, xxvii. 25.
 HARLAND, S. C., 1940: Polyploids in *Gossypium*. *Tropical Agriculture, Trinidad*, xvii. 53.
 HUSKINS, C. L., and SMITH, S. G., 1934: A Cytological Study of the Genus *Sorghum* Pers. II. The Meiotic Chromosomes. *Journ. Genetics*, xxviii. 387.
 HUTCHINSON, J. B., and STEPHENS, S. G., 1944: Progress Reports from Experiment Stations for 1942-3, p. 170, Empire Cotton Growing Corporation, London.
 SKOVSTED, A., 1937: Cytological Studies in Cotton. II. Chromosome Conjugation in Inter-specific Hybrids. *Journ. Genetics*, xxxiv. 97.
 STEPHENS, S. G., 1944: Phenogenetic Evidence for the Amphidiploid Origin of the New World Cottons. *Nature*, 153. 53.

EXPLANATION OF PLATE IV

Illustrating the paper by J. B. Hutchinson, S. G. Stephens, and K. S. Dodds on 'The Seed Hairs of *Gossypium*'.

Fig. 1. *G. raimondii*.

Fig. 2. *G. thurberi*.

Fig. 3. 2 (*G. arboreum* × *G. thurberi*.)

Fig. 4. *G. barbadense*.

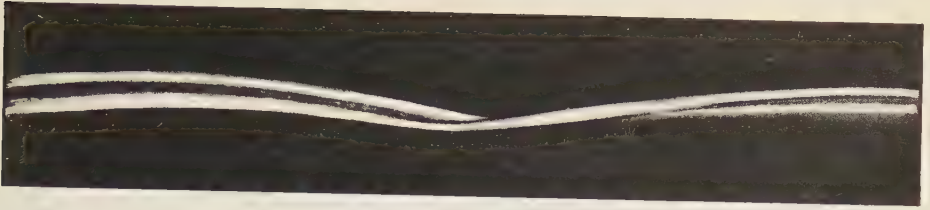


FIG. 1. *G. raimondii*



FIG. 2. *G. thurberi*



FIG. 3. 2 (*G. arboreum* × *G. thurberi*)

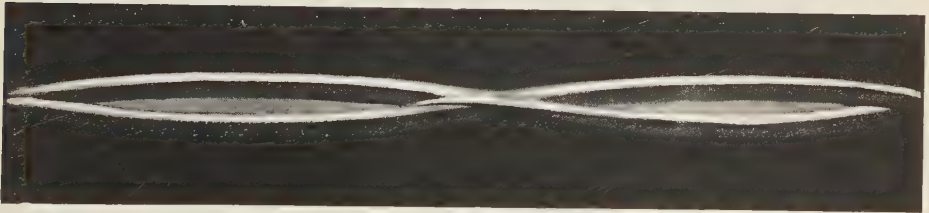


FIG. 4. *G. barbadense*

Studies in the Physiology of Leaf Growth

I. The Effect of Various Accessory Growth Factors on the Growth of the first Leaf of isolated Stem Tips of Rye

BY

R. S. DE ROPP

(Research Officer, Agricultural Research Council, attached to Rothamsted Experimental Station)

With three Figures in the Text

INTRODUCTION

IN the present series of investigations, by culturing excised stem tips from rye embryos on artificial media of known composition, information was sought regarding the factors which control the growth and differentiation of leaves. This form of culture makes possible a study of the factors concerned in leaf growth independently of their effect on the growth of roots. The culture of isolated stem tips was first attempted by Robbins (1922), who showed that shoot tips of peas and corn will grow to a limited extent in darkness provided that they are supplied with a source of carbohydrate. White (1933) maintained isolated stem tips of *Stellaria media* in hanging drops of a culture solution containing mineral salts, dextrose, and yeast extract. He was able to demonstrate that such fragments were capable of making a limited amount of further growth. They also had a capacity to differentiate fresh leaf primordia. The culture of isolated leaves was subsequently attempted by Bonner, Haagen-Smit, and Went (1939). These workers maintained immature leaves excised from etiolated pea plants in a synthetic medium containing mineral salts and sucrose. They showed that in such a medium these excised leaves were capable of making a limited amount of growth, which could be further increased by the addition to the medium of a diffusate obtained from peas. Their results led them to conclude that a non-specific leaf-growth hormone may exist which affects the growth of leaves in several species of plants. In this connexion Avery (1935) has stated also that a hormone, probably auxin *a*, is concerned in the leaf growth of *Nicotiana*.

Some further information regarding leaf growth and differentiation was provided by the investigations of White (1939) and Skoog (1944) of the behaviour of callus cultures of the hybrid *Nicotiana glauca* × *N. langsdorfii*. Both these workers were able to show that a change in physical conditions is by itself sufficient to initiate leaf differentiation in such cultures. They did not postulate the existence of any special leaf-growth-controlling hormone.

MATERIALS AND METHODS

Stem growing-points from the embryos of rye grains (var. Petkus) were removed with aseptic precautions and placed on nutrient agar. The position of these excised growing-points in the embryo is shown in Fig. 1. The first leaf primordium, to which attention was mainly directed, is an organ about

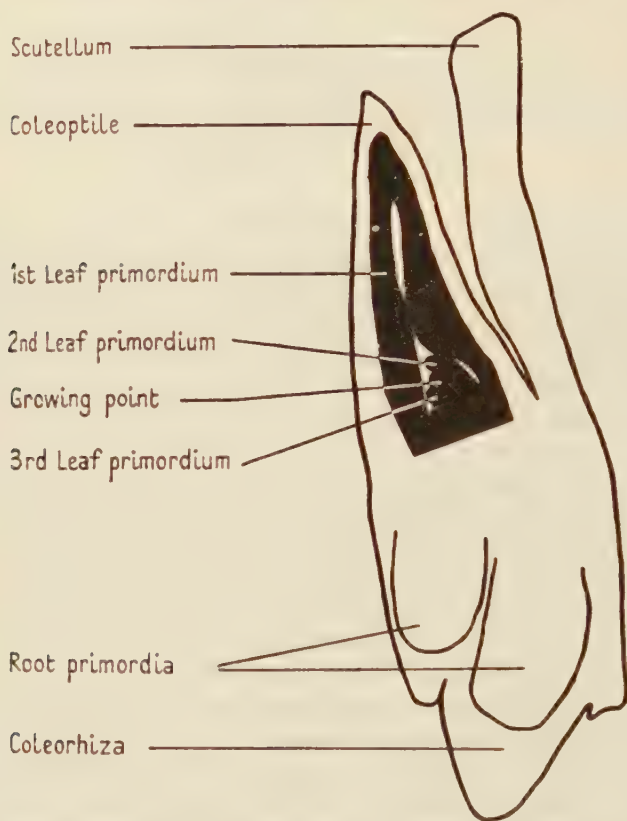


FIG. 1. Longitudinal section of a rye embryo before germination, showing (in black) the form of the stem tip removed in these experiments.

1 mm. long containing ten vascular bundles and enclosing two or three younger leaf primordia as well as the growing-point (Fig. 2).

It was found unnecessary to sterilize the grain before removal of the stem tips provided aseptic precautions were taken during that operation. All instruments used were sterilized by flaming alcohol. Contaminated cultures were rare, not more than 1 per cent., and were rejected. To facilitate the removal of stem tips the grain was soaked for about 30 minutes. Longer soaking rendered the primordium increasingly susceptible to injury. Shorter soaking times made difficult the process of stripping off the coleoptile. The first leaf primordium was very easily damaged and a very small amount of

injury entirely inhibited the growth of the organ. The actual excision was made with a flat-ground, sterile needle, the cut being made immediately beneath the point of attachment of the first leaf.

The medium, to which all accessory factors to be tested were added, was made up with distilled water, prepared in a Pyrex glass still, and contained 2 per cent. sucrose, complete mineral salts in the proportions suggested by White (1943), and 0.3 per cent. agar. The sucrose and mineral salts used were of 'Analar' standard of purity and the agar was extracted before use by the method recommended by Robbins (1939). Usually each isolated stem tip

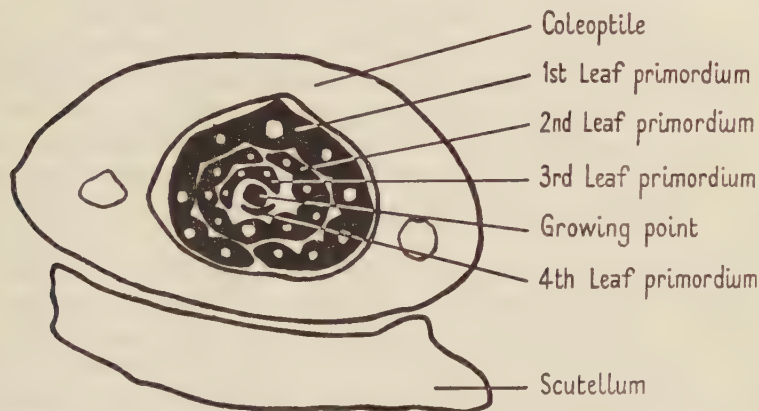


FIG. 2. Transverse section of a rye embryo at the level of attachment of the leaf primordia.

was cultured on 15 ml. of agar contained in a 50-ml. conical flask. The medium was autoclaved for one minute at 10 lb. pressure, which treatment was found to give adequate sterilization. Substances to be tested for their growth-promoting efficacy were added to the agar before or after sterilization according to whether they were heat sensitive or resistant.

The stem-tip cultures were incubated in a constant temperature chamber at 25° C. in complete darkness. In nearly all experiments the period of incubation was 2 weeks, at the end of which time the amount of growth of the first leaf was assessed by measuring its length, fresh weight, and dry weight. For each treatment there were twenty replicates, the minimum number from which a reasonably accurate estimate of the treatment effects could be obtained. White (1943) recommends the same number of replicates for experiments with isolated root tips. The standard errors worked out at about 10 per cent. of the means.

EXPERIMENTAL

1. *Growth of the first leaf on isolated and attached stem tips.*

This experiment was designed to provide a comparison of the growth rate of the first leaf when attached to: (a) an excised stem tip cultured on sucrose

mineral agar; (b) an excised whole embryo cultured on sucrose mineral agar; (c) an embryo in the intact grain cultured on plain mineral agar.

Groups of 120 excised stem tips, excised embryos, and intact grains were placed on the media described above and incubated in darkness at 25° C. From these groups 20 individuals were removed after the following periods of incubation: 3 days, 5 days, 1 week, 2 weeks, 4 weeks, and 6 weeks. The length and fresh weight of each individual was determined. Dry weights were estimated for groups of 5. From these values the means were calculated which, with their standard errors, are shown in Table I.

One of the most outstanding features of the behaviour of these stem-tip cultures was the absence of growth of any part of the tip except the first leaf. Whereas in the intact embryo growth of the second leaf began within 48 hours of germination, in stem-tip cultures the second leaf primordium remained in an embryonic condition, showing no signs of developing unless, by some chance, the stem tip differentiated a root. Whenever this happened it was accompanied by growth of the second leaf and by renewed meristematic activity of the entire stem tip. The nature of this interaction of stem and root is still obscure.

The quantitative differences in the growth of the first leaf under these different sets of circumstances are brought out in Table I. Length, fresh weight, and dry weight reach a maximum value in most cases after 7 days' incubation. But the amount of increase differs widely according to the condition of the stem tip to which the leaf was attached. If measurements at 0 and 7 days are compared for leaves under these different sets of conditions the ratios obtained are as follows:

	Isolated stem tip.	Isolated embryo.	Whole grain.
Length	18	122	187
Fresh weight	45	180	497
Dry weight	13	18	55

The effect on leaf growth of excising the stem tip may best be assessed by comparing ratios for leaves attached to isolated stem tips with those for leaves attached to isolated embryos. Such a comparison shows that excision of the stem tip resulted in a very considerable reduction of growth in leaf length and fresh weight. Dry-weight growth was reduced to a much smaller extent. The uptake of nutrients thus appears to have been much the same in both cases, the difference being in the uptake of water. A consideration of the values for leaves attached to the whole grain reveals that the presence of the endosperm resulted in a great increase in the uptake both of nutrients and of water by the leaf. Evidently the endosperm provided some factors necessary for leaf growth which were either absent from, or inadequately supplied by, the artificial medium.

Further differences in the behaviour of the three groups occurred when growth was studied for longer than seven days (Table I). Leaves attached to excised stem tips showed no significant change either in fresh or dry weight

TABLE I
Growth of (i) first Leaf attached to isolated Stem Tip, (ii) isolated Embryo, and (iii) intact Grain. Incubated in Darkness
at 25° C. (Means of 20 estimations)

Days	0	3	5	7	14	28	42
Isolated stem tip	Length (mm.)	5.68 ± 0.44	17.50 ± 0.88	18.11 ± 1.54	19.27 ± 1.29	15.70 ± 1.57	14.21 ± 1.58
	Fresh wt. (mg.)	2.10 ± 0.26	6.60 ± 0.49	11.15 ± 1.22	10.88 ± 0.88	11.10 ± 1.22	11.81 ± 1.88
	Dry wt. (mg.)	0.42 ± 0.05	1.10 ± 0.19	2.40 ± 0.23	2.00 ± 0.15	2.58 ± 0.11	2.37 ± 0.17
Isolated embryo	Length (mm.)	35.46 ± 1.72	88.92 ± 2.00	122.20 ± 4.00	136.42 ± 7.16	131.10 ± 5.30	131.80 ± 5.49
	Fresh wt. (mg.)	13.13 ± 1.14	34.46 ± 1.46	45.00 ± 2.50	46.42 ± 3.39	26.81 ± 3.31	17.21 ± 2.14
	Dry wt. (mg.)	1.20 ± 0.15	2.70 ± 0.20	3.17 ± 0.25	2.86 ± 0.18	2.56 ± 0.15	2.86 ± 0.17
Whole grain	Length (mm.)	37.81 ± 3.25	130.00 ± 1.73	187.21 ± 3.91	175.40 ± 6.70	195.61 ± 6.31	173.40 ± 5.39
	Fresh wt. (mg.)	18.45 ± 2.38	86.40 ± 1.78	124.20 ± 3.57	118.80 ± 8.80	62.01 ± 6.30	17.81 ± 1.81
	Dry wt. (mg.)	2.18 ± 0.32	7.00 ± 0.21	10.00 ± 0.30	7.00 ± 0.28	6.70 ± 0.27	6.00 ± 0.20

after the seventh day. The apparent decrease in length after the second week did not recur in other experiments. Such leaves remained alive, turgid, and capable of regenerating chlorophyll on exposure to light even after 8 weeks' incubation in darkness. Only after this time did they become brown and die. Leaves attached to isolated embryos were more short-lived. They began to lose water after the second week and were flaccid and dead by the sixth week. Leaves attached to intact grains had an even shorter life. Their dry weight fell rapidly after the first week, and excessive water loss was accompanied by shrinking and browning of the tissues. Within 4 weeks all leaves in this series were brown and dead.

In leaves attached to the intact embryos and grown in the dark, a red or yellow colouring matter developed which attained its maximum intensity about 7 days after germination and then gradually disappeared. In leaves attached to isolated stem tips this colour hardly appeared at all; a trace of yellow could sometimes be seen near the tips of such leaves but they were, for the most part, quite colourless. They also differed from the attached leaves in that only the lamina developed, the sheath being entirely absent.

2. *Effect of sucrose and mineral salts on the growth of the first leaf attached to an isolated stem tip.*

The two main components of the nutrient medium, sucrose and mineral salts, were tested separately and together to determine their effect on the growth of the first leaf in stem-tip cultures (Table II). In the absence of

TABLE II

Effect of Sucrose and Mineral Salts on Growth of first Leaf of isolated Stem Tip. Incubated 2 Weeks in Darkness at 25° C. (Means of 20 estimations)

	No sucrose or mineral salts.	Mineral salts alone.	Sucrose alone.	Sucrose plus mineral salts.
Length (mm.)	2.15 ± 0.04	2.26 ± 0.04	6.17 ± 0.53	18.73 ± 2.38
Fresh wt. (mg.)	0.45 ± 0.01	0.61 ± 0.02	4.02 ± 0.25	11.73 ± 1.00
Dry wt. (mg.)	0.10*	0.15*	0.71 ± 0.02	2.1 ± 0.15

* Replicates bulked before drying.

sucrose no growth could be observed whether mineral salts were supplied or not. When both were supplied together growth was significantly greater than it was in the presence of sucrose alone, a fact which indicates that a supply of carbohydrate was not by itself sufficient to ensure maximum growth. The different appearances of these stem-tip cultures grown on different media is shown in Fig. 3.

3. *Effects of crude plant extracts on growth of the first leaf attached to an isolated stem tip.*

The effect of plant extracts on the growth of isolated plant organs has been studied by several workers. Robbins and White (1937) recorded that an



FIG. 3. Effect of sucrose and mineral salts on the growth of isolated rye stem tips on artificial media. Top row, sucrose and mineral salts present; second row, mineral salts absent; third row, sucrose absent; bottom row, tips on removal from the grain. ($\times 2\frac{1}{2}$) (Photograph by V. Stansfield.)

extract of maize grain slightly increased the growth of isolated corn roots. Van Overbeck *et al.* (1944) have stated that a factor affecting the growth of isolated *Datura* embryos is present in coconut milk. The claims of Bonner, Haagen-Smit, and Went regarding the growth-promoting efficacy of pea

diffusate have been previously mentioned. The effect of an extract of yeast on root growth is well known from the work of White and others.

In the present work the following crude extracts were tested to determine their effect on the growth of the first leaf in isolated stem-tip cultures: (a) Pea diffusate prepared by the method described by Bonner, Haagen-Smit, and Went, added to agar, after autoclaving, to give a final concentration of 1 per cent. (b) Hot-water extracts of sprouted and unsprouted rye grain added to agar, after autoclaving, to give a concentration of 1 per cent. (c) Cold-water extracts similar to (b). The grain was ground and extracted with water at 1° C. for 24 hours, the sprouted grain having been first dried in the frozen state. This extract was sterilized by filtration and added to the agar just before it cooled to give a final concentration of 1 per cent. (d) Hot-water extract of dried yeast added to agar to give a final concentration of 1 per cent. (e) Endosperm digest, obtained by placing an excised fragment of endosperm, with the scutellum attached, on the surface of the nutrient agar. Such fragments were rendered sterile by carefully stripping them of the outer integuments. On the surface of the agar they underwent digestion by enzymes produced by the attached scutellum. The soluble products of this digestion could diffuse out into the agar on which an excised stem tip was then placed in the usual way.

This experiment was set up in three sections each having its own control, and the results are shown in Table III. It is clear that neither extracts nor endosperm digest had any effect on the growth of the first leaf attached to these stem tips. The differences between separate treatments are no greater than those between untreated controls. The pea diffusate, to which Bonner, Haagen-Smit, and Went were inclined to attribute a rather general leaf-growth-promoting efficacy, had no effect on this material.

TABLE III

Effect of Crude Plant Extracts on Growth of first Leaf attached to Isolated Stem Tip. Incubated in Darkness at 25° C. for 2 Weeks. (Means of 20 estimations)

Extract.	Length (mm.).	Fresh wt. (mg.).	Dry wt. (mg.).
Sprouted grain			
(Hot water extract)	16.31 ± 1.20	12.71 ± 1.41	2.44 ± 0.21
Unsprouted grain			
(Hot water extract)	15.98 ± 1.67	11.76 ± 1.13	2.45 ± 0.12
Yeast			
(Hot water extract)	13.01 ± 1.22	9.52 ± 0.81	2.00 ± 0.11
Control (no extract)	15.56 ± 1.11	9.81 ± 1.06	1.84 ± 0.14
Sprouted grain			
(Cold water extract)	15.43 ± 1.06	11.62 ± 1.32	2.40 ± 0.19
Unsprouted grain			
(Cold water extract)	19.21 ± 1.13	16.89 ± 1.20	2.05 ± 0.16
Endosperm digest	14.61 ± 1.31	10.22 ± 0.96	2.65 ± 0.14
Control (no extract)	17.26 ± 0.91	11.05 ± 0.81	2.21 ± 0.20
Pea diffusate	15.96 ± 1.72	10.10 ± 1.31	2.07 ± 0.13
Control (no diffusate)	14.98 ± 1.23	11.80 ± 1.14	2.71 ± 0.17

4. *Effect of vitamins of the 'B' groups on the growth of the first leaf attached to an isolated stem tip.*

Thiamin, nicotinic acid, and pyridoxine have all been shown to be necessary for the growth of isolated plant roots (White, 1937; Robbins and Schmidt, 1939; Bonner, 1943). Few investigators have concerned themselves with the possible role of these substances in leaf growth, though Bonner, Haagen-Smit, and Went (1939) tested the effects of thiamin, riboflavin, and biotin on the expansion of fragments of *Raphanus* leaves and found all of them without effect. In the present work thiamin, nicotinic acid, riboflavin, calcium pantothenate, pyridoxine, and biotin were each tested separately for their effect on the growth of isolated stem tips, and thiamin, pyridoxine, and nicotinic acid were tested in combination. The individual vitamins were added to sucrose mineral agar before autoclaving to give the following final concentrations (γ /c.c.):

Thiamin.	Nicotinic acid.	Riboflavin.	Ca pantothenate.	Pyridoxine.	Biotin.
0.5	0.1	0.1	0.1	0.1	0.1

The growth of the first leaf in stem-tip cultures containing these individual substances is shown in Table IV. Thiamin appears to have had a significant effect but this was not observed again in later experiments. None of the other vitamins had any effect.

TABLE IV

Effect of 'B' Vitamins on Growth of first Leaf attached to Isolated Stem Tip. Incubated in Darkness for 2 Weeks at 25°C. (Means of 20 estimations)

Vitamin.	Length (mm.).	Fresh wt. (mg.).	Dry wt. (mg.).
Thiamin	20.91 \pm 1.38	12.32 \pm 1.06	2.51 \pm 0.22
Nicotinic acid	19.65 \pm 1.67	11.37 \pm 1.32	2.00 \pm 0.11
Riboflavin	19.30 \pm 1.11	10.15 \pm 1.01	2.00 \pm 0.14
Ca pantothenate	19.37 \pm 1.06	9.06 \pm 0.86	1.75 \pm 0.16
Pyridoxine	15.35 \pm 0.91	10.00 \pm 1.73	2.06 \pm 0.20
Biotin	17.91 \pm 1.72	9.27 \pm 1.92	1.90 \pm 0.15
Thiamin, pyridoxine and nicotinic acid combined			
10 γ per c.c.	13.01 \pm 1.18	9.31 \pm 0.78	2.21 \pm 0.21
1 γ per c.c.	14.92 \pm 0.76	11.15 \pm 0.66	2.39 \pm 0.18
Control (no vitamin)	18.18 \pm 1.28	10.55 \pm 0.97	2.05 \pm 0.20

To test the effect of thiamin, nicotinic acid, and pyridoxine in combination the vitamins were added to nutrient agar to give final concentrations of 1, 10, and 100 γ per c.c. of each component. Growth was entirely inhibited by the highest concentrations of these substances. The lower concentrations produced no significant effect (Table IV).

5. *Effect of β indole acetic acid and α naphthalene acetic acid on growth of the first leaf attached to an isolated stem tip.*

The capacities of these substances to act as growth-promoting factors for plants have been variously reported by different workers. Smith (1940) has

stated that they inhibit leaf growth on excised stem tips of *Helianthus annuus*, and Ball (1944) has found that they produce abnormalities in leaf growth when applied to the attached shoot apex of *Tropaeolum majus*. Spoehr (1942) has shown that they have no growth-promoting effect on the leaves of albino maize plants. Avery (1935) has claimed that auxin is involved in the growth of leaves of *Nicotiana*, but the substance in question was more probably auxin *a* than either indole or naphthalene acetic acid. Berger and Avery (1944), however, have isolated indole acetic acid from a precursor in dormant maize grains which suggests that this substance may play a part in the growth of the embryo of the Gramineae.

In these experiments two concentrations of each acid were used. Weighed amounts of the dry substance were dissolved in small quantities of absolute alcohol, diluted with sterile water and added to the agar after autoclaving. The final concentrations of growth substance in the agar were 10^{-4} and 10^{-7} . The growth made by isolated stem tips cultured on this medium is shown in Table V. The higher concentration of growth substance inhibited growth entirely and no results have therefore been recorded. At the lower concentration they had no significant effect on the growth of the first leaf.

TABLE V

Effect of Various Accessory Factors on the Growth of the first Leaf attached to Isolated Stem Tip. Incubated for 2 Weeks in Darkness at 25° C. (Means of 20 observations)

Vitamin concentration.	Length (mm.).	Fresh wt. (mg.).	Dry wt. (mg.).
β indole acetic acid $1/10^7$	18.29 ± 1.16	9.88 ± 1.65	1.50 ± 0.18
α naphthalene acetic acid $1/10^7$	17.63 ± 1.01	11.47 ± 1.33	1.65 ± 0.20
Ascorbic acid $\left\{ \begin{array}{l} 100 \text{ } \gamma \text{ per c.c.} \\ 10 \text{ " " } \\ 1 \text{ " " } \end{array} \right.$	12.52 ± 1.50	8.82 ± 1.00	1.53 ± 0.25
	17.94 ± 1.24	10.22 ± 1.00	1.72 ± 0.25
	21.00 ± 2.00	11.68 ± 0.96	2.37 ± 0.27
Vitamin E	17.31 ± 1.71	10.31 ± 0.58	2.12 ± 0.20
Vitamin K	11.60 ± 1.56	8.93 ± 0.75	1.93 ± 0.14
Adenine	11.28 ± 1.54	10.42 ± 1.01	2.28 ± 0.13
Guanine	17.00 ± 2.56	12.42 ± 2.71	2.24 ± 0.21
Uric acid	16.87 ± 1.62	12.00 ± 2.00	2.50 ± 0.16
Caffein	14.36 ± 1.73	9.37 ± 1.02	1.87 ± 0.18
Control (nogrowthfactor)	17.17 ± 1.12	11.18 ± 2.01	2.14 ± 0.18

6. *Effect of ascorbic acid on growth of the first leaf attached to an isolated stem tip.*

Ascorbic acid has been shown to be present in relatively large quantities in practically all green leaves (Bessey and King, 1933) and its function has been associated with biological oxidation (Carrol, 1943). Havas (1935) has stated that the vitamin may function as a growth-promoting substance in wheat, but this statement lacks confirmation. Clark (1937) has found it to be without any growth-promoting effect on the *Avena* coleoptile.

In the present work recrystallized ascorbic acid was dissolved in distilled water, sterilized by filtration and added to nutrient agar after autoclaving to

give final concentrations of 1, 10, and 100 γ per c.c. No significant increases in growth were produced by the presence of this vitamin. At the highest concentration used it had a depressing effect on growth (Table V).

7. *Effect of vitamins E and K on the growth of the first leaf attached to an isolated stem tip.*

The presence of vitamin E (α tocopherol) in lettuce leaves and wheat-germ oil (Olcott and Mattill, 1934), and of vitamin K in green leaves of various plants (Dam and Nielsen, 1940), suggests that both these substances may play some role in leaf metabolism. An experiment was set up to determine whether either substance was capable of affecting the growth of the first leaf attached to an isolated stem tip. The vitamins were added to nutrient agar to give a final concentration of 10 γ per c.c. Vitamin E was dissolved in a small amount of alcohol before adding to the agar with which it formed a stable suspension. Vitamin K was added in the form of the water-soluble analogue 'Synkavit' (tetra sodium salt of 2-methyl-1:4-naphthohydroquinone diphosphate). Neither of these vitamins produced any effect on the growth of the first leaf under the conditions of the experiment (Table V).

8. *Effect of purine derivatives on the growth of the first leaf attached to an isolated stem tip.*

That certain purine derivatives, particularly adenine, are capable of increasing leaf growth has been claimed by Bonner and Haagen-Smit (1939), but the effects recorded by these workers were very small and appear of doubtful significance. In the present work the purine derivatives, adenine, guanine, caffeine, and uric acid were tested to determine their effect on the growth of rye leaves attached to isolated stem tips. They were added to the nutrient agar to give a concentration of 10 γ per c.c. None of these substances was capable of producing a significant increase in the growth of the first leaf under these conditions (Table V).

DISCUSSION

The experiments described in this paper show that, when the entire growing-point was excised from a rye embryo and cultured in darkness on 2 per cent. sucrose and mineral salts, a limited amount of growth resulted which was almost entirely confined to the first leaf. The growth of the first leaf primordium attached to the isolated stem tip differed in several respects from the growth of the primordium when attached to the embryo, whether the embryo was cultured on an artificial medium or attached to its own endosperm. These differences may be listed as follows: (1) smaller increase in fresh and dry weight and particularly in length; (2) absence of red or yellow pigment; (3) failure to form a leaf sheath.

It is clear from (1) that the isolated stem tip is able to utilize the nutrients supplied only to a limited extent. This might be due to the absence from the medium of some factor essential for growth, or to a physical inability on the

part of the stem tip to continue absorbing nutrients at a sufficient rate to maintain growth.

The nutrient medium used in this work supplied both major and minor mineral elements, carbon as sucrose and nitrogen as nitrate. It is possible that sucrose and nitrate were not the best forms in which to supply these essential nutrient elements, and that better growth would have been obtained with some other source of carbon and nitrogen. Possibly again some factor of a vitamin or hormone nature essential to the nutrition of the shoot was lacking from the nutrient solution. The present investigation was concerned with this aspect of the problem. Seven different crude plant extracts and thirteen pure substances were tested, but none enabled the isolated stem tip to make any greater growth than with sucrose and mineral elements alone.

Nevertheless, it would not be safe to conclude that none of the substances tested in these experiments plays any part in leaf growth. In all cases in which an isolated plant organ is grown on an artificial medium, interpretation of results is complicated by questions regarding the ability of such an organ to absorb all the nutrients it requires from the medium. The stem tip is not physiologically adapted to absorb already-elaborated nutrients direct from an external medium. Growth, therefore, may be limited by this simple disability.

Attention has already been drawn to the immediate effect on the growth of these isolated stem tips of the development of a root system. When such development occurs the meristematic activity of the entire growing point is awakened and new leaves are developed in which the leaf sheath is differentiated and yellow colouring matter formed. The nature of this stimulating action is at present unknown.

SUMMARY

The growth of isolated stem tips excised from rye embryos on a culture medium containing 2 per cent. sucrose and mineral salts was studied.

Growth on this medium was found to be almost entirely confined to the first leaf and presented several abnormal features. In the absence of mineral salts growth was much reduced. In the absence of sucrose no growth occurred.

The following substances added to the medium failed to produce any significant increase in leaf growth: (a) Crude extracts of peas, rye grains both sprouted and unsprouted, yeast, and a digest of rye endosperm; (b) the 'B' vitamins thiamin, nicotinic acid, calcium pantothenate, pyridoxine, riboflavin, biotin; also ascorbic acid, vitamins E and K, indole acetic and naphthalene acetic acids, and the purine derivatives adenine, guanine, uric acid, and caffeine.

It was observed that if any isolated stem tip developed a root the entire growing point was stimulated to meristematic activity, and leaves normal in form and size developed.

The author wishes to thank Dr. D. J. Watson for his criticism of this paper and Mr. S. French for technical assistance. He is indebted to Messrs. Roche Products, Ltd., for samples of α tocopherol, 'Synkavit', and calcium *d* pantothenate, and to Messrs. Glaxo Laboratories for a sample of pyridoxine and adenine.

LITERATURE CITED

- AVERY, G. S., 1935: Differential Distribution of a Phytohormone in the Developing Leaf of *Nicotiana* and its relation to Polarized Growth. Bull. Torrey Bot. Club, lxii. 313.
- BALL, E., 1944: The Effects of Synthetic Growth Substances on the Shoot Apex of *Tropaeolum majus*. Amer. Journ. Bot., xxxi. 316.
- BERGER, J., and AVERY, G. S., 1944: Isolation of an Auxin Precursor and an Auxin Indole-acetic Acid) from Maize. Amer. Journ. Bot., xxxi. 199.
- BESSEY, O. A., and KING, C. G., 1933: The Distribution of Vitamin C in Plant and Animal Tissues and its Determination. Journ. Biol. Chem., ciii. 687.
- BONNER, D. M., and HAAGEN-SMIT, A. J., 1939: Leaf Growth Factors. II. The Activity of Pure Substances in Leaf Growth. Proc. Nat. Acad. Sci., xxv. 184.
- , and WENT, F. W., 1939: Leaf Growth Hormones. I. A Bio-assay and Source for Leaf Growth Factors. Bot. Gaz., ci. 128.
- , 1943: Further Experiments on the Nutrition of Isolated Tomato Roots. Bull. Torrey Bot. Club, lxx. 184.
- CARROLL, G. H., 1943: The Role of Ascorbic Acid in Plant Nutrition. Bot. Rev., ix. 41.
- CLARK, W. G., 1937: Ascorbic Acid in the *Avena* Coleoptile. Bot. Gaz., xcix. 116.
- DAM, H., and NIELSEN, N., 1940: Weitere Untersuchungen ueber die Bildung und Bedeutung des Vitamin K in Pflanzenorganismus. Zeit. f. physiol. Chemie, cclxv. 80.
- HAVAS, L., 1935: Ascorbic Acid (vitamin C) and the Germination and Growth of Seedlings. Nature, cxxxvi. 435.
- OLCOTT, H. S., and MATTILL, H. A., 1934: Vitamin E. I. Some Chemical and Physiological Properties. Journ. Biol. Chem., civ. 423.
- OVERBEEK, J. VAN, SIU, R., and HAAGEN-SMIT, A. J., 1944: Factors affecting the Growth of *Datura* Embryos in vitro. Amer. Journ. Bot., xxxi. 219.
- ROBBINS, W. J., 1922: Cultivation of excised Root Tips and Stem Tips under sterile Conditions. Bot. Gaz., lxxiii. 376.
- , 1939: Growth Substances in Agar. Amer. Journ. Bot., xxvi. 772.
- , and SCHMIDT, M. B., 1939: Vitamin B₆, a Growth Substance for Excised Tomato Roots. Proc. Nat. Acad. Sci., xxv. 1.
- , and WHITE, W. B., 1937: Effect of Extracts from the Corn Plant on Growth of Excised Root Tips. Bot. Gaz., xcviii. 520.
- SKOOG, F., 1944: Growth and Organ Formation in Tobacco Tissue Cultures. Amer. Journ. Bot., xxxi. 19.
- SMITH, B. S., 1940: The Effect of Various Accessory Growth Substances on Excised Stem Tips of *Helianthus annuus* L. in Culture. Thesis. University of Michigan.
- SPOEHR, H. A., 1942: The Culture of Albino Maize. Plant Physiol., xvii. 397.
- WHITE, P. R., 1933: Plant Tissue Cultures. Results of Preliminary Experiments on the Cultivation of Isolated Stem Tips of *Stellaria media*. Protoplasma, xix. 97.
- , 1937: Vitamin B₁ in the Nutrition of Excised Tomato Roots. Plant Physiol., xii. 803.
- , 1939: Controlled Differentiation in a Plant Tissue Culture. Bull. Torrey Bot. Club, lxvi. 507.
- , 1943: A Handbook of Plant Tissue Culture. Lancaster, Pennsylvania.

Experimental and Analytical Studies of Pteridophytes

VI. Stelar Morphology: The Occurrence of Reduced and Discontinuous Vascular Systems in the Rhizome of *Onoclea sensibilis*

BY

C. W. WARDLAW

(Department of Cryptogamic Botany, University of Manchester)

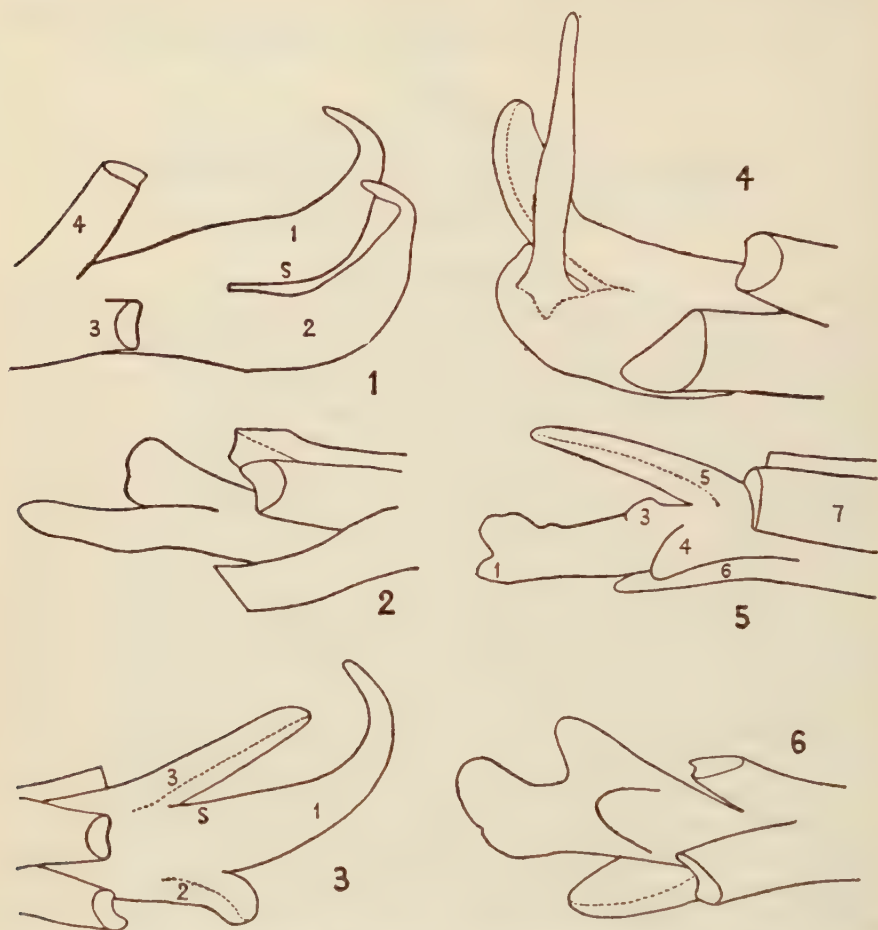
With Plates V and VI and thirty-three Figures in the Text

INTRODUCTION

THE shoot stele in pteridophytes is normally continuous from the base of the plant to a point immediately below the apical meristem; parenchymatous leaf-gaps and in some species perforations may occur at intervals, but these only occupy part of the cross-sectional area of the stele. Yet under certain abnormal conditions of growth it is conceivable that the differentiation of vascular tissue at the apex might not take place, parenchyma or some other tissue being formed instead. If on a return to more normal conditions the differentiation of vascular tissue was resumed, then the shoot stele would show a longitudinal interruption or discontinuity. Such a state of affairs, though rare in pteridophytes, is not unknown. Holloway (1939) has described large prothalli of *Psilotum triquetrum* in which a discontinuous vascular strand is present. The formation of this strand takes place during periods of active growth at the apical meristem: during periods of relative inactivity, only distended parenchymatous tissue is formed and the apical meristem itself becomes partly parenchymatous. On a return to active growth vascular tissue is again differentiated. Hence, over the length of the prothallus an intermittent vascular strand is to be observed. Discontinuities in the vascular system of ferns occur where arrested buds or detached meristems situated in adult regions of the shoot have been induced to develop; indeed, every stage between complete confluence of the bud stele with the vascular system of the shoot and complete separation from it may be observed (Wardlaw, 1943, 1943a, 1944). In such instances the discontinuity is between the product of a lateral meristem and that of the main-shoot meristem, whereas in *Psilotum* it is the central vascular strand which is affected. Such variability in the formation of vascular tissue points to the variability and morphogenetic plasticity of the growing region.

In the present paper an account is given of some unusual developments found in experimentally treated rhizomes of *Onoclea sensibilis*: the reduced and discontinuous vascular systems observed are of a kind which, so far as the writer is aware, have not hitherto been described. The specimens also provide

new evidence on regressive changes in leaf development. An analysis of such materials in conjunction with a study of the normal development should contribute towards an understanding of morphogenetic processes at the growing-point.



TEXT-FIGS. 1-6. The terminal region of a number of greatly reduced plants of *Onoclea sensibilis*. The awl-like leaves are numbered in basipetal sequence. S, approximate position of inactive shoot apex. ($\times 6.5$.)

MATERIALS

When the rhizome apex of *Onoclea sensibilis* is removed, plantlings originate from detached meristems at intervals along the rhizome (Wardlaw, 1943a). During the summer months the growth of plantlings is rapid, the elongating horizontal shoot bearing a number of small adult leaves. These wither down in autumn. In one experiment plantlings of this kind were kept during the winter months in an incubator at 22° – 25° C., with ordinary daylight illumina-

tion and at a high relative humidity. Growth at a high temperature and with feeble illumination was thus enforced during what is normally a period of quiescence. Under these conditions the rhizomes elongated and produced new leaves, but these became progressively smaller till they had the character of juvenile leaves, i.e. those characteristic of the young sporophyte. By February of the following year juvenile leaves were no longer being formed but only swollen leaf primordia. The rhizome apices, which were no longer covered with the usual investment of scales, terminated either in a bulbous mass of tissue or in an awl-like extension (Text-figs. 1-6). If, in Text-fig. 1, the two curved awl-like projections are interpreted as being modified leaf primordia, then no shoot apex is apparent. Similarly, in Text-figs. 3 and 4 the morphological category of the terminal organ of the shoot cannot be decided by external inspection. In Text-fig. 2 the last outstanding leaf is a fleshy spine-like object, while the shoot apex is bulbous and swollen. Various swollen leaf primordia are also to be seen in Text-figs. 5 and 6, the terminal region of the shoot being again large and distended. In normal plants of this age the leaf primordia develop into small adult leaves while the shoot, which is protected by curled leaf primordia and scales, terminates in a small conical meristem.

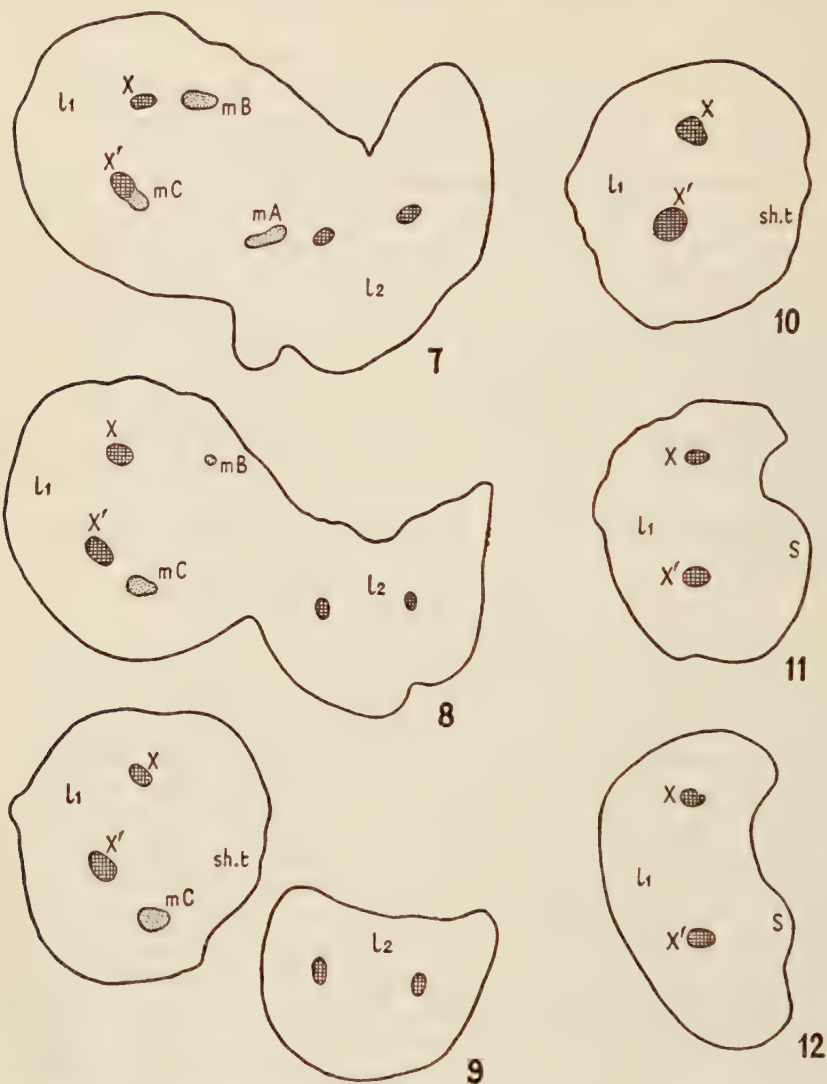
These abnormal plants were fixed and investigated by means of microtome serial sections.

INTERNAL MORPHOLOGY

The internal structure of the plant shown in Text-fig. 1 is illustrated diagrammatically in Text-figs. 7-12. These show transverse sections of the rhizome in acropetal sequence. The two terminal organs are seen to be swollen leaf primordia, each having the pair of vascular strands characteristic of *Onoclea*. The question thus arises as to the fate of the shoot meristem.

At a lower level in the shoot than that shown in Text-fig. 7 the vascular system consists of a normal dictyostele with typical binary leaf-traces. Text-fig. 7 shows the arrangement of the vascular tissue just below the seeming bifurcation. The right-hand side of the section, consisting of a leaf-base with two strands, can be identified with the outgrowth indicated as leaf 2 in Text-fig. 1; these strands become conjoined with two meristeles of the shoot lower down. The centre of Text-fig. 7 is occupied by the shoot, three meristeles being present; to the two on the left the strands of leaf 1 in Text-fig. 1 are becoming conjoined. Text-fig. 8 shows the distribution of vascular tissue near the point of bifurcation, the outline of leaf 2 being now quite characteristic. The notable point, however, is that one of the shoot meristeles (*mA*) has gradually diminished in size and faded out; while a second meristele (*mB*) is apparently doing likewise. Text-fig. 9, taken just above the point of bifurcation, shows leaf 2 on the right hand and a leaf-shoot construction on the left. In the latter, only one meristele (*mC*) of the original three meristeles now remains, together with the two strands of leaf 1. Higher up, as shown in Text-fig. 10, the third meristele has also faded

out, the binary leaf-trace being now the only vascular tissue to be seen in the section. The outline of this section is not that of a typical leaf-base; the right-



TEXT-FIGS. 7-12. Transverse sections in acropetal sequence of the plant illustrated in Text-fig. 1. The axial meristemes (*mA*, *mB*, *mC*) fade out. The inactive shoot meristem (*S*) has been carried forward on the adaxial surface of the youngest leaf (*l*₁). *x*, *x'*, *y*, *y'*, binary leaf-traces of the two leaves; *sh.t.*, shoot tissue. ($\times 22$.)

hand side, in fact, still consists of shoot tissue, but there is no axial vascular tissue. Still higher up, the conjoint leaf-shoot nature of leaf 1 (*l*₁) remains apparent, the typical leaf outline becoming more evident as the shoot tissue diminishes. Finally, at the level shown in Text-fig. 12, a typical leaf cross-

section is seen, the small projecting mass of tissue (*s*) being all that is left of the drawn-out terminal region of the shoot (Pl. V, Figs. 6, 7).

The tissue marked (*s*) in Text-figs. 11 and 12 had the appearance and characteristic staining of meristematic or semi-meristematic tissue. The parenchymatous cells of the leaf region contained large numbers of starch grains, whereas those of the residual meristematic shoot region did not. The cells at the junction of the two regions contained small starch grains (Pl. V, Figs. 6, 7; Pl. VI, Fig. 9). In this plant, if meristematic activity had been resumed at the residual shoot apex, the new vascular tissue formed would not have been continuous with that in the older region of the shoot. These observations show that leaf 1 in Text-fig. 1 is a composite structure in its proximal region but foliar in its distal region.

An examination of the object illustrated in Text-fig. 3 has shown the terminal awl-like organ to be a composite leaf-shoot structure in its basal region and a foliar structure in the distal region. At the level where the stem component began to taper out, a progressive dwindling and disappearance of the vascular strands of the shoot was also observed. This specimen therefore showed the same essential features as those described above for Text-fig. 1.

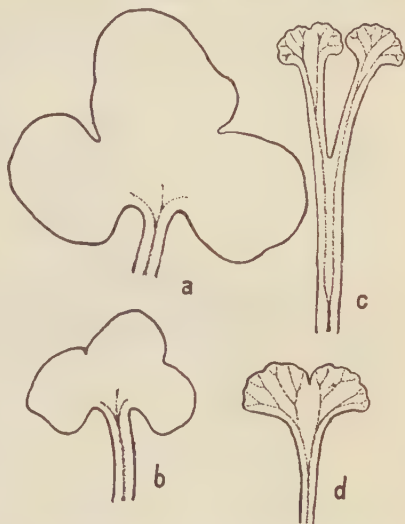
Of these several specimens, that illustrated in Text-fig. 5 showed the greatest variability in the organization of its vascular system. Diagrammatic illustrations of the internal structure at different levels from the proximal to the distal region are shown in Text-figs. 13–22 and in Pl. VI, Figs. 10–15. The leaf numbers correspond with those indicated in Text-fig. 5. At the level of attachment of leaf 4 the vascular system of the shoot was approximately normal, the leaf gaps and positions of older leaves being indicated in Text-fig. 13. Above this level, with the ‘closing’ of the gaps of the older leaves, the shoot stele became solenostelic, the single wide gap relating to leaf 4 in Text-fig. 14. (In that abbreviated, swollen leaf-primordium two vascular strands were present in the basal region but only a single strand nearer the apex.) Higher up, the gap of leaf 4 (*l*₄) closed, but a wide gap opened on the opposite side in relation to the insertion of leaves 3 and 2 (Text-fig. 15). Leaf 3 is seen in Text-fig. 5 as a small swollen protuberance; leaf 2, situated on the underside, was almost inhibited in its development. Above the insertion of leaf 2 the shoot stele underwent a remarkable diminution in size; at first its outline in transverse section was irregular but soon it became cylindrical and protostelic (Text-figs. 16 and 17; Pl. VI, Fig. 10). This protostele became still smaller, particularly in the region of conjunction with the leaf-trace of leaf 1 (*l*₁). Text-fig. 18 shows that of the two vascular strands, the leaf-trace (*l*₁) is considerably larger than the shoot stele (*st*); also Pl. II, Figs. 11, 12. Immediately above this level the shoot stele practically disappeared (Text-fig. 19; Pl. II, Figs. 13, 14); it consisted of two or three narrow cells enclosed within an endodermis, and was so inconspicuous as almost to escape observation. Near the distal end of the shoot the shoot stele underwent enlargement (Text-figs. 20, 21; Pl. II, Fig. 15), and could be followed right up to the terminal meristem.

were removed from one of the shanks, the other being allowed to grow on untouched. Both shanks underwent considerable extension. The leaves became progressively smaller until eventually only juvenile leaves, i.e. those characteristic of the young sporophyte, were being produced (Text-fig. 23). Towards the end of the experimental period the untouched shank again bifurcated (Text-fig. 24); one shank (l_1 in Text-fig. 24) terminated in what appeared to be a fasciated shoot bearing two swollen leaf primordia and a small terminal bud; the other shank (l_2), which also had the appearance of a fasciated shoot, terminated in two juvenile leaves, with two lateral buds (S_1 and S_2) situated at the base of the two petioles. The other shank of the first dichotomy, which had been consistently defoliated, produced a number of juvenile leaves and eventually terminated in an awl-like organ.

Text-figs. 25–32 show the essential features of the bifurcating shank illustrated in Text-fig. 24. Below the bifurcation a normal dictyostele was present. The disposition of individual strands near the region of dichotomy was also normal (Text-fig. 25). A leaf occupied a position at right angles to the dichotomy—a common feature in ferns—while in the two shanks leaf-traces ($x, x^1; y, y^1$) were present. Near the point of dichotomy the shoot meristeles faded out (Text-figs. 25, 26). Just above the point of separation each of the two shanks had approximately the outline of a leaf-base with its leaf-trace (Text-fig. 27), but, as a full examination shows, each was a composite structure consisting of leaf-base and reduced shoot.

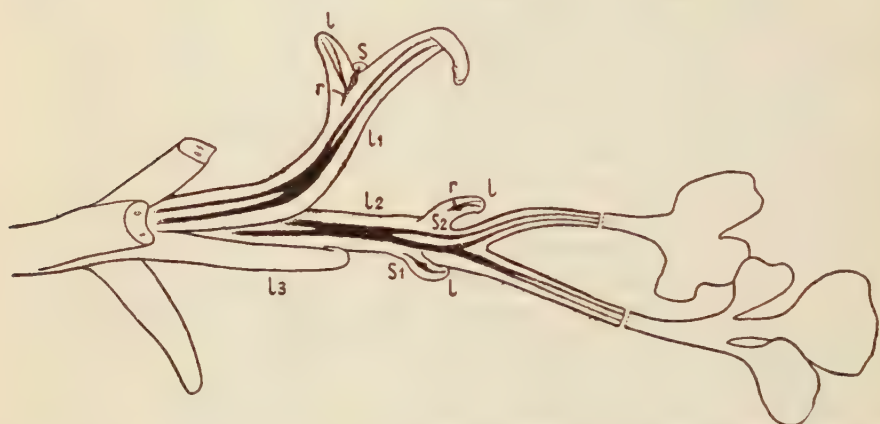
In the smaller shank (l_1 in Text-fig. 24) the vascular strands coalesce and then separate into two strands again. Near the distal end (Text-figs. 24, 28, 29) a bud originates in what appears to be the outer cortex of the leaf. This bud has an atypical meristem (S) consisting of semi-meristematic or parenchymatous cells and has given rise to a leaf (l) and a root-trace (r). The vascular system of the bud is not connected with the leaf-trace or with the shoot stele below.

Somewhat similar observations have been made on the larger shank of the dichotomy (Text-fig. 24, l_2). The meristeles become conjoined then separate again, the two terminal leaves having each two vascular strands (Text-figs. 24, 32). Two lateral buds (S_1, S_2), each of which consists of a leaf and a diminutive shoot, have no vascular connexion with the leaf-traces already described (Text-figs. 30, 31).



TEXT-FIG. 23. Leaves from reduced plants showing regression. *a, b*, small adult leaves; *c, d*, juvenile type of leaf. ($\times 3.5$.)

The shank of the first dichotomy which had been subjected to continuous defoliation showed normal dictyostely and phyllotaxis in the proximal region. In the distal region, however, as illustrated in Pl. V, Figs. 1-5, the shoot meristeles faded out and the terminal region consisted of an awl-shaped organ. In the latter the binary leaf-trace was the conspicuous feature in the basal region, but near the distal end a small bud with a protostelic vascular strand and a root-trace was present. This terminal shoot stele was not connected with either of the original leaf-trace strands.



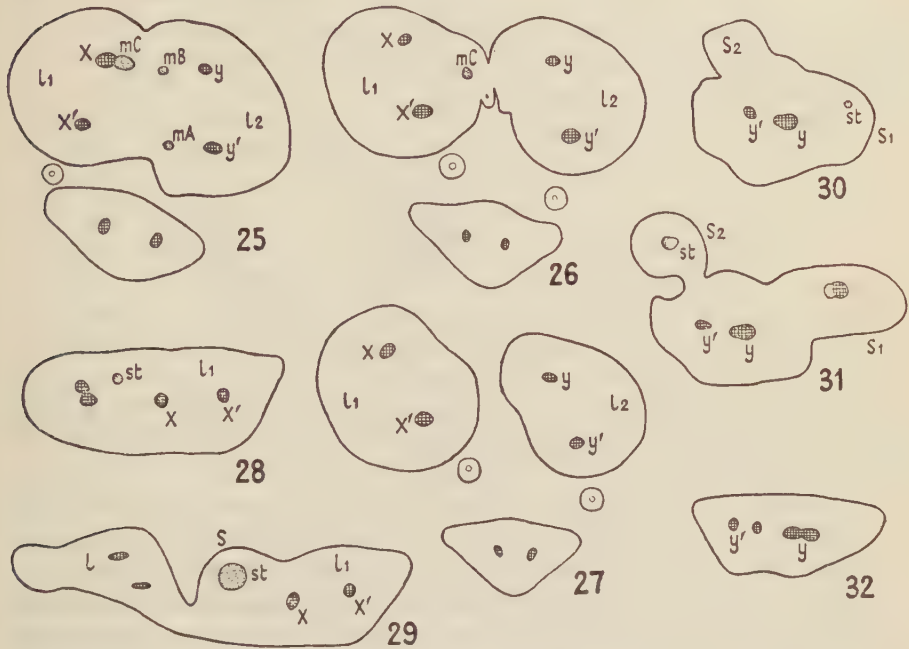
TEXT-FIG. 24. Terminal region of a greatly reduced bifurcating shoot. Vascular tissue is stippled. l_1, l_2, l_3 , terminal leaves in basipetal sequence; s, s_1, s_2 , shoot apices; l , leaf of small shoot; r , root-trace. For description, see Text. ($\times 2.3$.)

These several observations are interpreted as indicating a state of affairs in which the shoot apex became attenuated and temporarily non-functional. During this inactive phase it was carried forward by the elongation of the adjacent petiole base; practically no axial tissue was formed and the shoot stele faded out. Later, when growth was resumed by the reduced shoot apex, a small leafy bud (with a small shoot stele, leaf- and root-traces) made its appearance. Such materials thus show a longitudinal interruption or discontinuity of the axial stele.

THE EFFECT OF CONTINUOUS DEFOLIATION

In the literature of pteridophytes occasional references have been made to more or less notable morphological changes induced by growth under 'starvation' conditions (Thompson, 1915; Goebel, 1908). In those instances where the materials have not been produced under experimental conditions the factors involved in the abnormal development cannot be known. Diminished bulk, slow development, &c., are the usual indications that some interference with 'normal' growth has taken place. The abnormal specimens described in the previous sections might be considered to be the result of

growth under 'starvation' conditions. The question then arises as to the precise nature of this 'starvation'. The conditions provided, the diminished bulk of the plants, and the absence of leaf laminae suggest that lack of sufficient carbohydrate may have been the factor limiting growth. Starch grains were, however, abundantly present throughout the terminal regions and could be observed on the margin of the residual shoot meristems. An exception



TEXT-FIGS. 25-32. Transverse sections in acropetal sequence of the reduced bifurcating shoot illustrated in Text-fig. 24. Figs. 25, 26, 27, near point of bifurcation: the shoot meristemes *mA*, *mB*, *mC* are fading out, leaving the binary leaf-traces *x*, *x'*, and *y*, *y'*. Figs. 28, 29, sections through the distal region of leaf 1 (*l*₁) showing a shoot stele (*st*) and a leaf (*l*), as well as the leaf-trace *x*, *x'*. Figs. 30, 31, sections through the base of the bifurcating region of leaf 2 (*l*₂), showing the two lateral shoots, *s*₁, *s*₂. Fig. 32, section of the bifurcating petiole above the buds. (x 9.)

to this was noted in the instance of a defoliated shoot, sections of which are illustrated in Pl. V, Figs. 1-5: starch was present in the more proximal region of the shoot but was not observed in the distal region.

The idea that a deficiency of carbohydrate is directly associated with the remarkable changes described above is one which can be tested by experimental treatment, i.e. by observation of the results when all new leaves are removed as soon as they are about to unroll and become photosynthetically active. There is a difficulty in that most ferns have considerable starch deposits in the rhizome and fleshy leaf bases; these can be drawn upon for growth and development at the apex. Thus even though constant defoliation

is practised, the suggestion that carbohydrate 'starvation' is involved must be accepted with caution.

Pieces of rhizome of *Onoclea sensibilis*, 7 cm. long, with intact apices, were trimmed so that all the old leaf-bases and roots were removed. These materials were placed on moist peat in an illuminated incubator maintained at 22°–25° C. and at a high humidity. As the experiment was begun in October, conditions were by no means optimal for growth: temperature and humidity were too high and the illumination too feeble. Pieces of rhizome (*A*) were kept continuously defoliated, i.e. all leaves about to unroll were cut off; similar pieces (*B*) had the older foliage leaves removed only; the control pieces (*C*) were left untouched.



TEXT-FIG. 33. Greatly reduced shoot resulting from continuous defoliation. *b*, lateral buds (see Text). The bifurcated distal region of the same shoot with juvenile leaves five months later is shown in the upper figure. ($\times 0.65$.)

The week-to-week observations need not be detailed here. When the experiment was concluded at the end of 11 months, the following points were noted in the plants (*A*) which had been continuously defoliated: (i) the rhizomes had undergone rapid elongation and a considerable reduction in diameter; (ii) new leaves continued to be produced and there was an abundant root development; in some specimens many lateral buds developed more or less simultaneously (Text-fig. 33); (iii) the new leaves were of the lobed, dichotomous or fan-shaped type, like the 'juvenile' leaves of the young sporophyte; (iv) some of the plants had dichotomized, with equal development of both shanks; (v) on microscopic examination, no trace of starch was found throughout the rhizomes; tracheides were only slightly lignified in the shoot stele but more so in the leaf-traces; (vi) despite the marked decrease in diameter in the distal region of these experimental shoots the phyllotaxis and dictyostelic condition were those of a normal adult rhizome. In brief, the removal of the source of carbohydrate was not attended by any marked morphological changes. Material (*B*) produced small adult leaves at the beginning, but also showed leaf regression: by April a few juvenile leaves were being unrolled but thereafter the effect of increased length of day was apparent, small adult leaves being developed. These plants showed no comparable elongation of the rhizome but dichotomy was frequent. The occasional lateral buds which appeared were arrested in their further development. These plants showed no abnormal features; starch was present along

the length of the rhizome. The control plants (C) developed approximately normal adult leaves and, from January to April, some small ones; the rhizomes dichotomized but produced no lateral buds and showed no marked elongation. After eleven months small adult leaves were still being produced. There were no abnormal developments. Starch was present along the length of the rhizome. Other defoliation experiments have yielded similar data.

DISCUSSION

The development of the young sporophyte to the adult is usually attended by a notable increase in structural complexity. During this process the characters by which the species can be recognized gradually become apparent, the shoot apex being the region in which this progressive elaboration or organization takes place. But the 'normal' development may be only one of several possible developments. Thus, in the present investigation, the normal 'upward' or progressive development to be seen in the growth of the young sporophyte to the adult has been reversed. Under certain conditions stout adult shoots have undergone a marked regression. Successive leaves have undergone reduction from the complex adult condition to the simple outline of juvenile leaves. Although, in extreme cases, all traces of the lamina had disappeared, the dorsiventral symmetry of these organs was still apparent. As far as it goes, such an observation would not support views as to the cladode origin of the pteridophyte megaphyll. In these materials, the vascular system has also shown notable regressive changes, the normal dictyostele being replaced by a solenostele and finally by a protostele.

Evidence of this nature does not support the theory of recapitulation, i.e. that the developmental history of the individual is a recapitulation of the history of the race. On the contrary it suggests that the characteristic morphology to be observed at successive stages during development is the result of interaction between the specific hereditary constitution and the prevailing external and internal conditions. In particular, the nutritional status of the growing point is of fundamental importance. In this view the simple outline and small size of the first leaves of the young sporophyte are directly related to the relatively limited supplies of nutriment from the parent prothallus. But, as the data of the present investigation show, such leaves are not restricted to the young sporophyte: they may be produced in adult shoots under experimental conditions. The view of Goebel (1900) that the primary leaves of the fern sporeling are strictly comparable in structure with the adult leaves but that their development has been arrested is supported by the present observations. Goebel (1908) has also induced regression in leaf form, e.g. in *Ceratopteris thalictroides* by culturing isolated stem apices.

That the attenuated condition of the specimens illustrated in Text-figs. 1-6 is not due to a shortage of carbohydrate has been made clear, abundant starch deposits in close proximity to the meristematic tissues having been observed. Moreover, as root development has been profuse, it seems improbable that

development has been limited by a shortage of mineral nutrients. The factors inimical to 'normal' growth probably include the high temperature and feeble illumination to which the plants were subjected. Basically, the regressive changes are referable to a curtailment of meristematic activity. Thus, new leaf primordia ceased to be formed (Text-figs. 1-6) and the existing primordia, instead of continuing to develop as a result of apical growth, became short, distended, awl-like parenchymatous organs. Apart from this failure to produce leaf primordia the shoot meristems showed other indications of arrested and anomalous growth. Now, continued growth at the apex of shoot or leaf implies continuous protein-synthesis, i.e. in the formation of protoplasm. In the plants under consideration, the attenuated condition may perhaps be referred to a deficiency of one or other of the constituent metabolites or activators necessary for protein synthesis. This metabolite may become limiting because of the high rate of utilization under the prevailing conditions of temperature: alternatively, in relation to the feeble illumination provided, the metabolite, if it is a product of photosynthesis, may not be produced in sufficient quantities for normal growth. The possibility that inhibiting agents are present should also be considered.

The attenuated shoots which have been described raise many points of interest. Under the experimental conditions provided, a parenchymatous development tends to replace the normal organization of the apical meristem, so that only a residue of semi-meristematic tissue may remain. A concomitant condition is seen in the fading out of the vascular strands, thereby affording a parallel instance to the discontinuous vascular system in *Psilotum triquetrum* already noted. Such observations support the hypothesis that the initial differentiation of vascular tissue in pteridophytes is directly related to the diffusion of a substance from the active meristem (Wardlaw, 1944).

In several instances the development of a leaf primordium has been associated with a more or less complete cessation of shoot growth. Such instances may appear to support the view that the leaf is the dominant feature of the leaf-shoot system and that the shoot stele is largely foliar in origin, i.e. constructed of decurrent leaf-traces, and only to a minor extent, if at all, of cauline or axial origin. In other words, in these reduced specimens of *Onoclea sensibilis* the kind of vascular system observed in the young plants of certain eusporangiate ferns has been, to some extent, reproduced. Thus in the Marattiaceae and Ophioglossaceae, Campbell (1921) has shown that the first few leaves of the young sporophyte apparently develop without any true or recognizable shoot apex being present; the initial vascular system of the shoot being apparently or actually a composite structure composed of fused, decurrent leaf-traces. So too with certain other ferns. Campbell has therefore been led to contemplate the sporeling plant 'as being composed of leaves as primary organs, and the so-called stem as being formed by the coalescence of leaf-bases' (Bower, 1923, p. 139). In the light of the new observations presented here it seems not improbable that, in these eusporangiate sporeling plants, a small, semi-inactive shoot apex may, in fact, be present, and that

it only becomes actively functional when certain products of photosynthesis begin to arrive in effective concentrations from the young leaves. It may perhaps be noted that in these young plants the leaves conform to a regular phyllotactic system, in precisely the same manner as in species where an active shoot apex is present from the outset. Another instance of active leaf development in conjunction with suppressed shoot activity (with temporary absence of a shoot vascular system) is seen in the young sporophytic plants of *Lycopodium cernuum*, *L. laterale*, &c. In these species the so-called 'protocorm' is a fleshy organ bearing vasculated leaves, which arise without apparent relation to a recognizable shoot apex. After some time, when a functional shoot apex can be recognized, a vasculated leafy shoot is produced. In these and many other instances the young sporophytes are growing either under xerophytic or epiphytic conditions or under conditions involving mycorrhizic nutrition. In the writer's view the anomalous embryonic developments which have been recorded should be interpreted in terms of nutritional factors: phyletic considerations, on which so much emphasis has hitherto been laid, should be viewed with caution.

In some ferns, e.g. the 'starved' specimen of *Deparia Moorei* described by Thompson (1915) and further discussed by Bower (1923), the dimensions of the leaf-gaps are diminished and the dictyostelic condition is replaced by a solenostelic condition. A somewhat parallel development has apparently taken place in the specimen illustrated in Text-fig. 5. In both instances the morphogenetic plasticity of the growing point and the importance of nutritional factors are indicated.

Some curious deviations from the normal development of the shoot of *Osmunda regalis* have been described by Lang (1924). In some respects these resemble the specimens of *Onoclea sensibilis* described above, particularly that figured in Text-fig. 24. 'The chief deviations from the normal (in *Osmunda regalis*) concern the branching of the shoot, the development of buds on the leaves of young plants, the transformation of the distal portion of leaves into shoots and in other cases the transformation of the distal portions of leaves into prothalli' (p. 53). Lang considers that these several abnormalities are referable to disturbances at the juvenile stage in plants of normal type. The appearance of the plants was suggestive of unfavourable nutrition. In this connexion it may be noted that the plants were forced in a heated greenhouse during the normal resting period, and that illumination was feeble. These conditions are closely comparable with those under which the reduced plants of *Onoclea sensibilis* were obtained. In *Osmunda* juvenile leaves and leaves of a still more rudimentary nature were produced. There was also evidence of the direct transformation of some of the leaf rudiments into shoots and the development of shoot buds at various levels on the highly modified leaf rudiments. The awl-like leaves of *Onoclea sensibilis* described above bear a close resemblance to these greatly reduced leaves, 'cylindrical dark green growths without a lamina', described for *Osmunda regalis*. But whereas in *O. regalis* 'the cylindrical green equivalents of leaves may pass directly into a

bud by forming a regular succession of leaves that develop normally', in *Onoclea sensibilis* the original shoot apex has been shown to be implicated in the subsequent development of petiole buds. Although it may for a time lose its formative capacity and become longitudinally extended along the base of the last formed leaf its identity is not lost. The modified shoot apex has, for example, been traced to a position some distance along the leaf-base or petiole as an isolated mass of semi-meristematic tissue. When conditions permit of a renewal of growth this inconspicuous mass of tissue may again give rise to a small shoot bud. Such buds will appear to be of adventitious origin, because of their position on the subtending petiole and their dissociation from the original shoot lower down. In this connexion, reference may be made to the very remarkable position occupied by the buds of *Dryopteris filix-mas*, i.e. high up on the abaxial side of the petiole: it has now been shown that these buds originate in an approximately axillary position on the surface of the shoot and that their displacement can be referred to the distribution of growth (Wardlaw, 1943). In *Osmunda regalis* the formation of buds may take place laterally on the rudimentary leaves, and in the axils of leaves, as well as at the extreme tip of the cylindrical leaf rudiments. It may be that there, too, the displacement of potentially meristematic tissue during growth is involved, but evidence is required. The alternative—that an actual transformation of a leaf rudiment into a shoot has taken place—is a very unusual phenomenon. Even in the most reduced leaf rudiments observed in *Onoclea sensibilis* there was no evidence of the transformation of the dorsiventral symmetry of the leaf into the characteristic radial symmetry of the shoot. As Lang himself pointed out, a more detailed account of the *Osmunda* materials is required. He further indicated the interest which attaches to such materials as an experimental means of interpreting the organization of the leafy shoot.

SUMMARY

An account is given of some curious deviations from the normal development shown by the rhizome of *Onoclea sensibilis* grown in a feebly illuminated incubator during the normal resting period. The rhizomes became thin and elongated, the new leaves progressively smaller until they consisted of non-laminate swollen, awl-like organs, and the shoot apex became inactive or intermittently inactive.

These plants showed greatly reduced and discontinuous vascular systems.

Under the conditions stated, the normal 'upward' or progressive development to be seen in the growth of the young sporophyte to the adult is reversed; the inferences to be drawn from this observation are discussed with special reference to the activity of the apical meristem.

There are clear indications that the attenuated or 'starved' condition of these reduced plants is not due to a shortage of carbohydrate.

LITERATURE CITED

- BOWER, F. O., 1923: The Ferns, vol. i. Cambridge.
 CAMPBELL, D. H., 1921: The Eusporangiate Ferns and the Stelar Theory. *Amer. Journ. Bot.*, viii. 303.
 GOEBEL, K., 1900: *Organography of Plants*. (English transl. by I. B. Balfour) Oxford.
 — 1908: *Einleitung in die experimentelle Morphologie der Pflanzen*. Leipzig.
 HOLLOWAY, J. E., 1939: The Gametophyte, Embryo, and young Rhizome of *Psilotum triquetrum* Swartz. *Ann. Bot., N.S.*, iii. 324.
 LANG, W. H., 1924: On some Deviations from the Normal Morphology of the Shoot in *Osmunda regalis*. *Mem. and Proc. Manchester Lit. and Phil. Soc.*, lxxviii. 53.
 THOMPSON, J. McL., 1915: *Deparia Moorei*. *Phil. Trans. Roy. Soc. Edin.*, i. 26.
 WARDLAW, C. W., 1943: Experimental and Analytical Studies of Pteridophytes. I. Development of Buds on the Rhizome of the Ostrich Fern. *Ann. Bot., N.S.*, vii. 171.
 — 1943a: II. Experimental Observations on the Development of Buds in *Onoclea sensibilis* and in Species of *Dryopteris*. *Ann. Bot., N.S.*, vii. 357.
 — 1944: III. Stelar Morphology: The Initial Differentiation of Vascular Tissue. *Ann. Bot., N.S.*, viii. 173.

DESCRIPTION OF FIGURES IN PLATES V & VI

Illustrating Professor C. W. Wardlaw's paper on The Occurrence of Reduced and Discontinuous Vascular Systems in *Onoclea sensibilis*.

All figures are from untouched photographs.

PLATE V

Figs. 1-5. Transverse sections in acropetal sequence of a greatly reduced plant showing the fading out of the axial meristeles, and the reappearance of an axial protostele in the distal region. Fig. 2: the last axial meristele is fading out, leaving two leaf-trace strands (left). Fig. 3: only the leaf-trace strands are present; the tissue on the right-hand side of the section is probably shoot tissue. Figs. 4, 5: as well as the leaf-trace (below) an axial protostele (with a root-trace) can be seen (above). ($\times 50$.)

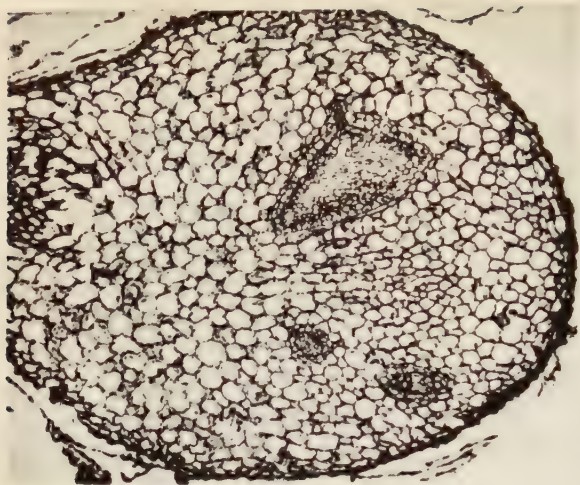
Figs. 6, 7. Transverse sections in acropetal sequence of the plant illustrated in Text-fig. 1. Fig. 6: the attenuated or reduced shoot is seen as a mass of tissue on the adaxial side of the leaf. Fig. 7: The inactive shoot meristem appears as a slightly projecting mass of tissue on the adaxial surface of the leaf. See Text and Text-figs. 7-12. ($\times 50$.)

Fig. 8. Transverse section of the reduced plant illustrated in Text-fig. 24, near the point of bifurcation. The axial meristeles are fading out. In each petiole-like shank a leaf-trace can be seen. See Text and Text-figs. 25-7. ($\times 30$.)

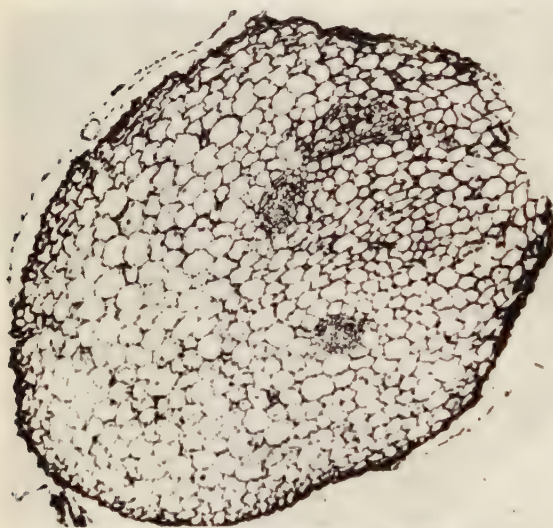
PLATE VI

Fig. 9. Companion section to Fig. 7 stained with iodine. Starch is abundantly present in the parenchyma lying in proximity to the inactive shoot meristem (above). ($\times 125$.)

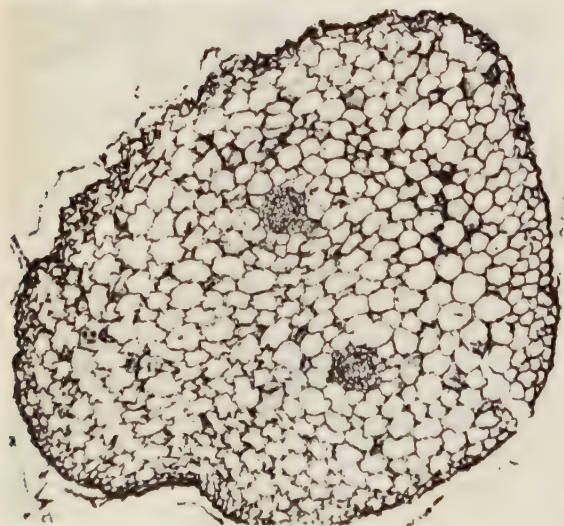
Figs. 10-15. Transverse sections in acropetal sequence of the plant illustrated in Text-figs. 5 and 13-22. Fig. 10: the protostele to which the axial dictyostele has been reduced. Figs. 11, 12: 'departure' of a leaf-trace (below) from the small shoot stele (above). Figs. 13, 14: the conspicuous stele is the leaf-trace: the shoot stele (above) has almost disappeared and consists of a few thin-walled elements only. Fig. 15, near the apex: the shoot stele has enlarged. ($\times 125$.)



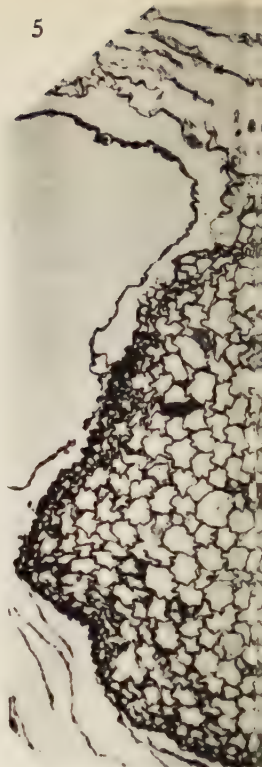
1



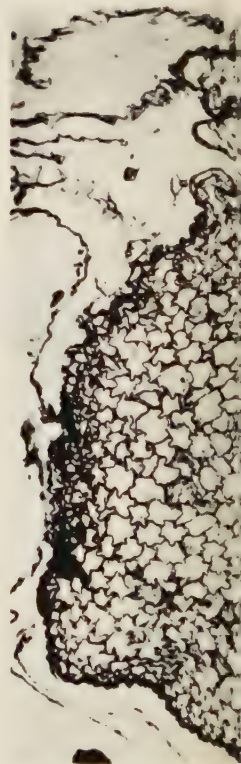
2

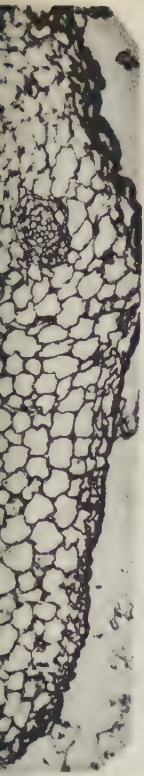


3

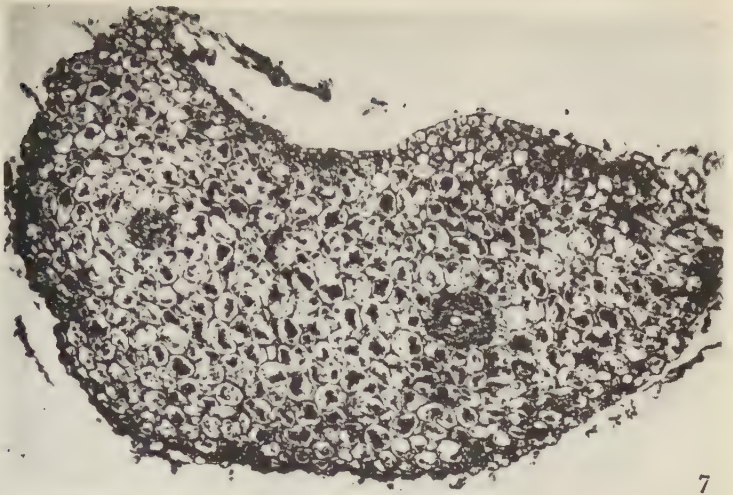


5

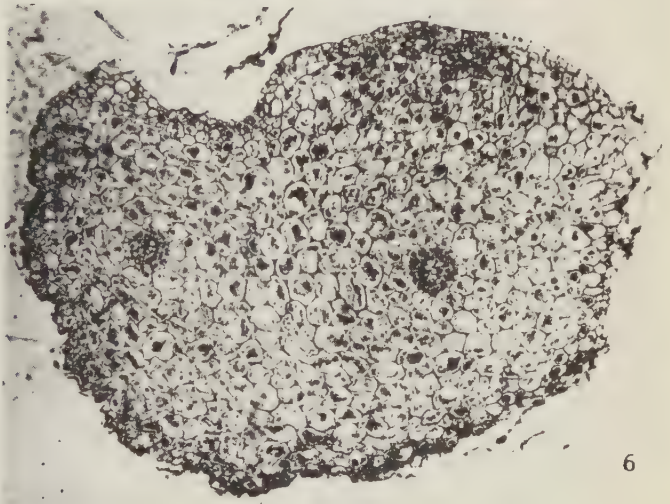




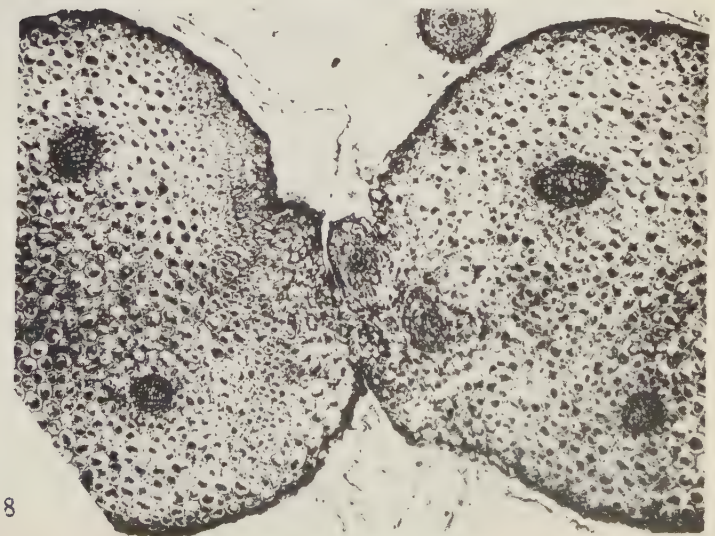
4



7

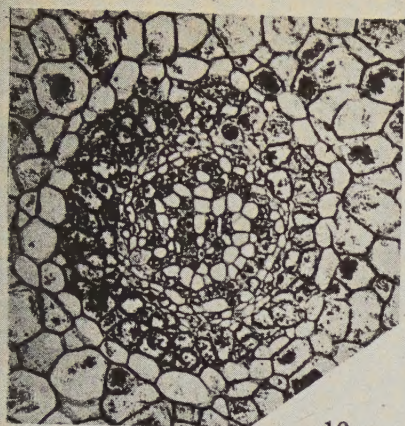


6

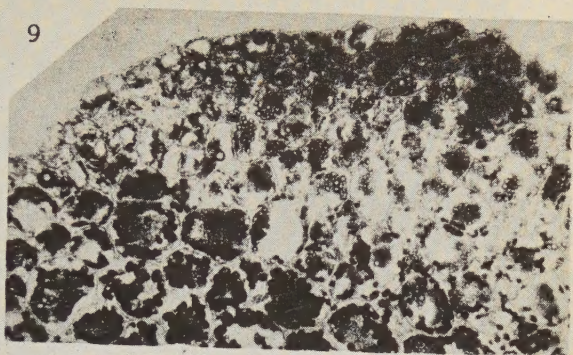


8

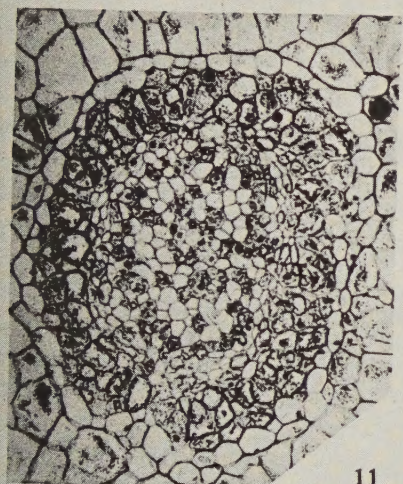
Huth coll



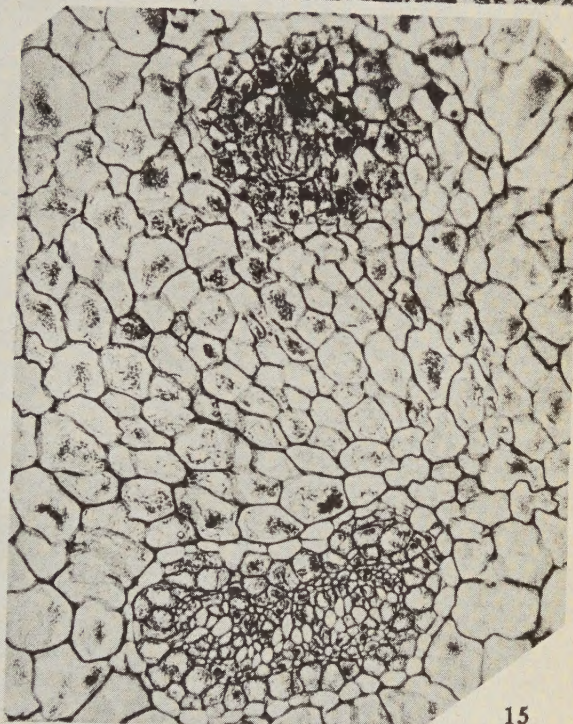
10



9



11



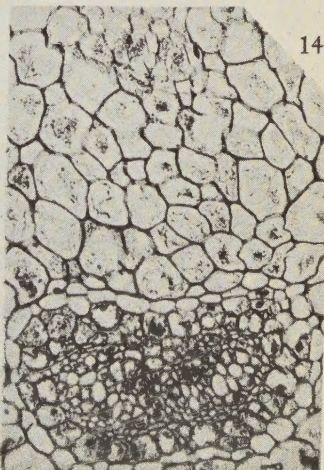
15



12



13



14

